SYSTEMATICS AND BIOGEOGRAPHY OF LEAFHOPPERS IN MADAGASCAR

BY

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DISSERTATION

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ABSTRACT

The biodiversity of Madagascar is well appreciated due to its high level of species richness and endemism. With more natural habitat being destroyed due to colonization and fulfilling essential needs of humans, presently it is considered as one of the priority hotspots for biodiversity conservation. Despite being the most diverse invertebrates in Madagascar, several insect families remain poorly known. Cicadellidae, one of the largest insect families distributed worldwide is one among them.

Prior knowledge of leafhopper fauna of Madagascar is based on only five previously published taxonomic studies and very limited sampling. No attempt using phylogenetic methods has been made to understand relationships among the leafhopper genera and to test the monophyly of previously recognized endemic Malagasy taxa. Owing to their Mesozoic origin, moderate dispersal abilities, and spectacular radiation in both New and Old World tropics, leafhoppers are excellent models for biogeographic and evolutionary studies related to the origins of the Malagasy fauna. A recent terrestrial arthropod inventory project in Madagascar conducted by the California Academy of Sciences yielded >60,000 ethanol-preserved leafhopper specimens, including representatives of most previously recorded species as well as many new species. This new material provided an opportunity to examine the phylogeny and biogeography of Malagasy leafhoppers in much greater detail than ever before possible. Specimens of three leafhopper subfamilies, Iassinae, Idiocerinae, and Cicadellinae were selected for study based on their independent evolutionary origins, according to a recently published family-level phylogeny, and their high diversity in samples used in the study.

The main objective of this study was to understand the historical biogeography of leafhoppers in Madagascar based on phylogeny. Phylogenetic relationships of the Malagasy fauna of the three selected leafhopper subfamilies to those of other continents were estimated using phylogenetic analyses of DNA sequence data. The phylogenies of these lineages were then used to estimate divergence times in order to test different biogeographic hypotheses and determine the most likely scenario(s) for the origin of the Malagasy leafhopper fauna. The species of two widely distributed leafhopper groups in Madagascar, the endemic idiocerine genus, *Nesocerus* and the subfamily Mileewinae, were also revised taxonomically and keys, descriptions and illustrations are provided.

Based on the phylogenetic informativeness within each lineage, different domains of 28S ribosomal RNA (D2, D6, D8, D9, D10), Histone (H3), 12SrRNA (12S) and Cytochrome Oxidase II were sequenced. Phylogenetic analyses based on Parsimony, Maximum Likelihood and Bayesian approaches recovered congruent topologies for each dataset. In Iassinae, analysis of a combined dataset of 28S, histone H3 and 12S nucleotide sequences, Malagasy endemic tribe Platyjassini was recovered as monophyletic and consistently placed as one of the deeper nodes within the subfamily. This suggests that this tribe belongs to an ancient lineage that became isolated and diversified within Madagascar. In Idiocerinae, analysis based on 28S, histone H3 dataset indicated that the Malagasy idiocerine fauna is polyphyletic, with different groups derived from either Asian or African genera, except one endemic genus, *Nesocerus*, recovered as sister to the rest of the subfamily. Phylogenetic analyses of Cicadellinae based on the D8 region of 28S, COII and Histone recovered the Malagasy fauna in two main clades, one including the genus *Madicola* and the other including the remaining genera. These to clades were arranged in a paraphyletic grade between the more basal (non-Malagasy) Old World Cicadellinae and the more derived New World fauna.

Bayesian divergence time analyses suggest that a combination of Cretaceous vicariance and Cenozoic dispersal shaped the biogeographic history of leafhoppers in Madagascar. The closest non-Malagasy relatives of the Madagascar endemic taxa Platyjassini (Iassinae) and *Nesocerus* (Idiocerinae) remain unclear. However based on the divergence time estimates, the timing of origin of these groups occurred during Cretaceous when Madagascar was joined with the Seychelles-Indian block and after becoming isolated, these groups underwent extensive diversification within Madagascar. In Idiocerinae, multiple dispersals from Asia and Africa were revealed to have given rise to the remaining Malagasy genera. The split of the Malagasy cicadelline fauna from other Old World Cicadellinae coincided with the period of existence of the Indo-Madagascar block, which may account for the early divergence of this group. The crown age of one of the clades of Malagasy Cicadellinae that includes genera distributed in eastern rainforests is estimated to be around early Eocene (~59 mya). This suggests that the group radiated within Madagascar soon after the emergence of eastern rainforests. An ‘Out of Madagascar’ dispersal to the New World through North Atlantic connections was also hypothesized as the most plausible scenario based on the timing of the split between the Malagasy lineage and the New World fauna.

Taxonomic revision of the endemic idiocerine genus *Nesocerus* revealed 29 new species. Morphological phylogenetic analysis of this genus supported the monophyly of this group and recovered two sister clades. Species belonging to one of the clades are widely distributed in eastern humid forests whereas the members of the other clade are mostly restricted to western dry forests. Revision of the subfamily Mileewinae revealed seven new species of *Ujna* from Madagascar. Species of this genus are placed into three species groups based on morphological characters. Utility of female genitalia characters in taxonomy of this subfamily is reported for the first time.

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TO

My husband, Madhu

&

My parents, Krishnankutty and Manu Bhai

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Chapter 1

Introduction

Madagascar is well known as a biodiversity hotspot (Myers *et al*., 2000), with high levels of species richness and endemism (80+%). Key factors believed responsible for the biodiversity of Madagascar are the comparatively large size of this tropical island, its long period of isolation, and relatively late colonization by humans (Goodman & Benstead, 2003). The recent compilation on biodiversity of Madagascar "*The Natural History of Madagascar"* with contributions from more than 300 researchers across different taxa, provides needed insight into the biodiversity of Madagascar, but also highlights glaring gaps in knowledge of the Malagasy fauna (Goodman & Benstead, 2005).

Insects are the most diverse invertebrates in Madagascar. During the mid twentieth century, several taxonomic studies of Malagasy insects were published, but these focused on a few families in various insect orders. (Goodman & Benstead, 2003). However, several insect families, irrespective of their worldwide richness and ecological importance, remain poorly known. **Leafhoppers** belonging to family Cicadellidae, one of the largest insect families with >22,000 described species distributed worldwide, is one among them. These exclusively plant feeding insects are abundant in both forests and grasslands (Morris, 1971; Hamilton, 1996), and are an important component of terrestrial food webs (Nickel, 2003). Despite their ecological importance, knowledge of the leafhopper fauna of Madagascar is rudimentary. Prior to this study, only five substantial works have been published on leafhoppers from the Malagasy region (Evans 1953, 1959; Freytag & Knight, 1966; Young, 1986; Dworakowska, 1997), and these give only a glimpse of the rich diversity and endemicity of leafhoppers in Madagascar. Apart from these taxonomic works, no attempt has ever been made to investigate the relationships between the Malagasy leafhopper fauna and the faunas of other continents using phylogenetic analysis.

Geological history of Madagascar.

In general, the origins of Madagascar biotic communities have long intrigued biogeographers due to its long history of isolation and unique biota. This tropical island began its journey to its current state of isolation in the Indian Ocean shortly after the separation of northern

(Laurasia) and southern (Gondwana) elements of supercontinent Pangea. Gondwana was a single landmass comprising what would become Africa, South America, Antarctica, Australia, India and Madagascar. Eastern (Madagascar plus India, Australia and Antarctica) and western Gondwana (Africa plus South America) separated around 165-155 mya. During this time, Madagascar along with the rest of eastern Gondwana began to drift southward relative to Africa. Although separation from Africa began as early as 165 mya, Indo-Madagascar-Antarctica and western Gondwana remained attached for about 20 million years during which biotic exchange would have taken place. Soon after the final separation of Indo-Madagascar from Africa, Antarctica and Australia moved southwards from Indo-Madagascar (Briggs, 2003). Separation of Madagascar from the Seychelles-Indian block commenced with northeastern movement of the latter that occurred around 88-63 mya (Storey *et al.,* 1995). Madagascar reached its current location in the Indian Ocean, approximately 400 km east of continental Africa between 130-118 mya.

Biogeographical history of Malagasy biota

Several studies have suggested complex patterns of biogeographic histories for various Malagasy taxa. Conclusions have differed with respect to the roles of Gondwanan vicariance versus Cenozoic dispersal. With respect to affinity of the Malagasy flora/fauna to fauna of other continents, three main patterns (affinities) have been proposed.

1) **Affinity with African mainland biota:** This appears to be the most favored current explanation for the origin of most Malagasy biota. Most of the studies on plants and vertebrates indicate that the sister groups of numerous endemic clades of Malagasy taxa are African taxa (Yoder & Nowak, 2006). Most animal studies have focused on vertebrates. Vences (2004) analyzed phylogenetic patterns of Malagasy amphibians, reptiles and other non-flying vertebrates and hypothesized transoceanic dispersal of ancestors from Africa to the west coast of Madagascar during the Cenozoic and, in a second step, subsequent species-rich radiations of certain lineages into the eastern rainforests. Regarding the mechanism of dispersal, both exchange through the Davie Ridge, a land bridge connection that existed in the Mozambique Channel between Africa and Madagascar, and the 'sweepstakes' hypothesis by G. G. Simpson 2(1940) have been proposed. The movement of fauna through the Davie Ridge has been considered the most logical explanation although it has some weaknesses. One of its main flaws

is that if exchange happened through a land connection, unrelated lineages would have moved simultaneously, which is not the case, and also timing of arrival to Madagascar would have coincided up to the maximum period of existence of the land bridge, but available data do not support either scenario. The second mechanism, 'sweepstakes' hypothesis, according to which the Malagasy fauna rafted from Africa, remained controversial and considered as a least plausible explanation for biotic interchange until the study by Ali & Huber (2010). The main criticism of this hypothesis was that the present, westward flow of ocean current (surface-water flow) in the southwest Indian Ocean from Madagascar to Africa would not allow rafting from Africa to Madagascar. Ali &Huber (2010), based on paleogeographic reconstruction and paleo-oceanographic modeling, showed that the direction of surface-water flow was opposite in this part of the Indian Ocean, i.e., eastbound, during the Paleogene period, exactly as required by a 'sweepstakes' process that allowed rafting. Subsequently the ocean current direction changed to the present day pattern.

2) Affinity with South American biota: South American affinity of Malagasy fauna based on a Late Cretaceous vicariant event has been reported for some vertebrates (Noonan and Chippendale, 2006). **S**ubaerial contacts between Antarctica and South America in the west and between Antarctica and IndoMadagascar in the east (Kerguelen Plateau and Gunnerus Ridge) have been proposed to exist until around 80 mya (Late Cretaceous) (Krause *et al*., 1997; Hay *et al.,* 1999). This 'Southern route' (fig. 1A) has been supported based on studies of both fossil (Evans *et al*., 2008) and extant taxa (Noonan and Chippendale, 2006) of vertebrates. A frog fossil recently discovered in Madagascar has closest modern–day relatives in South America (Evans *et al.,* 2008).

3) Affinity with Asian biota: A few studies suggested a scenario of dispersal from Asia rather than from Africa (Jansa *et al.*, 1999; Raxworthy *et al.,* 2002). Both transoceanic dispersal during the Early Tertiary (Schatz 1996) and vicariance during the Cretaceous have been proposed as possible mechanisms for Asian affinity. With respect to Late Cretaceous vicariance, a 'northern route' hypothesis (Rage, 1996) has been proposed based on vertebrate studies suggesting a terrestrial route from Asia to Madagascar via India and Seychelles Plateau during the Late Cretaceous (fig. 1B). Proponents of this hypothesis also suggest that this route may have existed after the time period during which the southern route was present. Discovery in Madagascar of a Late Cretaceous tooth fossil of a placental mammal that was otherwise restricted to Laurasia

supports biogeographical affinity with Asia (Krause, 2001; Averianov *et al.*, 2003). Geological evidence, mainly rearrangement of south Asiatic blocks (Jaeger *et al.*, 1989) and subduction of Indian continental lithosphere beneath Asia (Chemenda *et al.*, 2000) during the Late Cretaceous also suggests a possible terrestrial link between Asia and Madagascar.

Historical biogeographic patterns in Madagascar have been studied mainly in vertebrates. Although insects are the most diverse invertebrates in Madagascar, little is known about their origin and evolutionary history. Biogeographical origins of only a few groups of Malagasy insects have been posited. A few groups of stoneflies, dipterans, aphids, and earwigs are considered to be relicts of Gondwanan vicariance (Popham, 2000; Paulian & Viette, 2003). Fisher (1997, 2003) hypothesized an Afrotropical origin of Malagasy ants, one of the well-studied groups. However, these studies were based on distribution patterns only and did not incorporate phylogenetic information or estimates of the ages of the taxa, except a few recent ones. A few studies that included phylogenetic information inferred single or multiple transoceanic dispersals between Madagascar and other landmasses (Kerdelhue *et al.*, 1999; Fuller *et al.*, 2005; Monaghan *et al.*, 2005; Wirta *et al.*, 2008, Griswold *et al.*, 2012). Phylogenetic studies of Malagasy butterflies suggest a complex mix of Cretaceous vicariance followed by endemic species radiation within Madagascar, and subsequent dispersal from Madagascar to other areas (Torres *et al.*, 2001; Zhakarov *et al.*, 2004). A recent study of crayfish based on both phylogeny and divergence date estimation suggests Gondwanan vicariance of the Malagasy crayfish fauna (Toon *et al.*, 2010).

Leafhoppers: An excellent model for biogeographic studies

Owing to their Mesozoic origin, moderate dispersal abilities, spectacular radiation in both New and Old World tropics, and presence of endemic clades restricted to particular biogeographic regions, leafhoppers are excellent models for biogeographic and evolutionary studies related to the origins of the Malagasy fauna. A recent terrestrial arthropod inventory project in Madagascar conducted by the California Academy of Sciences has yielded >60,000 ethanol-preserved leafhopper specimens, including representatives of most previously recorded species as well as many new species. This new material provided an opportunity to examine the phylogeny and biogeography of Malagasy leafhoppers in much greater detail than ever before

possible. Large numbers of ethanol-preserved leafhoppers from other parts of the world, including Africa, Asia, and South America, were also included and this material facilitated global-scale phylogenetic and biogeographic studies of leafhoppers in this study. Specimens of three leafhopper subfamilies, **Iassinae, Idiocerinae, and Cicadellinae** were selected for my dissertation research because: 1) they are diverse monophyletic groups distributed worldwide (Nielson & Knight 2000; Dietrich *et al.*, 2001); 2) they represent independent evolutionary lineages; and 3) they are well represented in samples from all parts of Madagascar. Thus, their phylogenies should be informative of biogeographic patterns within Madagascar and between Madagascar and other continents. Phylogeny of these three focal leafhopper lineages was used to estimate divergence times to test the three main biogeographic hypotheses (listed under 'biogeographic history of Malagasy biota')

OBJECTIVES:

My dissertation includes four main objectives: **I)** phylogenetic analysis of the relationships of the Malagasy fauna of three leafhopper subfamilies Iassinae, Idiocerinae and Cicadellinae to those of other continents using morphological and molecular data; **II)** estimate times of origin of the Malagasy lineages using phylogenetic dating techniques to determine the most likely scenario(s) for the origin of the Malagasy leafhopper fauna; **III)** taxonomic revision of the endemic idiocerine genus *Nesocerus* from Madagascar; **IV)** taxonomic revision of tribe Mileewini from Madagascar.

Leafhoppers belonging to subfamily Iassinae are mostly arboreal and are distributed worldwide but most of the tribes and genera are restricted to a single biogeographic realm. This subfamily comprises over 2000 species and 146 genera currently placed in eight tribes. In Madagascar, this subfamily is represented mainly by two genera, *Platyjassus*, the only genus belonging to the endemic tribe Platyjassini, and *Batracomorphus*, a large and widespread Old World genus presently included in tribe Iassini. Evans (1953, 1959) described the monobasic Platyjassini and included four species. Linnavuori and Quartau (1975) noted the external similarity of Platyjassini with other tribes, Australian Reuplemmelini and New World Gyponini, but no attempt has been made so far to elucidate phylogenetic relationships of these taxa. Relatedness of Platyjassini with other tribes based on phylogeny and divergence time estimates is presented in **Chapter 2.** This chapter also discusses results of phylogenetic and biogeographic

analyses, using morphological and molecular data, of the whole subfamily Iassinae including all known tribes.

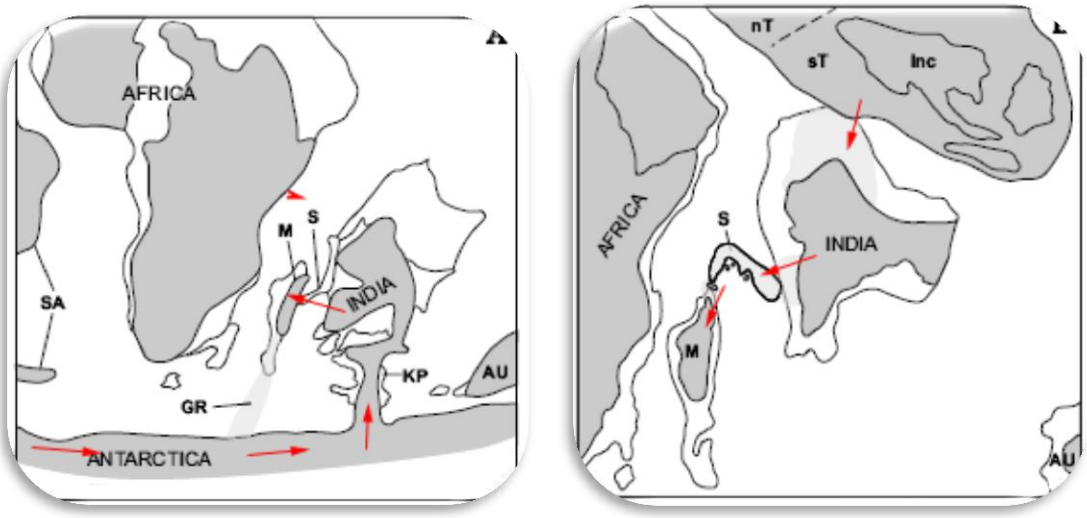
Idiocerinae are short, broad-headed, wedge-shaped leafhoppers, distributed worldwide. This subfamily comprises over 750 species (Zhang & Viraktamath, 2009). Only two works have been published so far on the Malagasy idiocerine fauna (Freytag & Knight 1966; Freytag & Cwikla 1984), which presently comprises 18 described species grouped into 4 genera, *Idiocerus*, *Idioscopus, Nesocerus* and *Kopamerra*. **Chapter 3** discusses phylogenetic relatedness of the Malagasy idiocerine fauna with that in other continents and the biogeographical scenario that shaped their present distribution in this island. Among the four described genera, *Nesocerus* is endemic to Madagascar, presently comprising eight described species. Although Freytag & Knight (1966) mentioned this genus as being related to *Idiocerus*, its phylogenetic relationships are unclear. Examination of leafhopper specimens from recently obtained samples revealed many new species of this genus. **Chapter 5** presents results of a taxonomic study of *Nesocerus* as well as a phylogenetic analysis of relationships among its species based on morphological characters.

Cicadellinae are xylem-feeding leafhoppers that have a worldwide distribution. These leafhoppers are brightly colored and widely distributed in Madagascar, but with maximum diversity in eastern humid forests. Young (1986) revised the Old World Cicadellinae, including the Malagasy fauna, which comprises 53 species in 27 genera; of which 25 are endemic. Madagascar has more cicadelline genera than mainland Africa (seven genera) despite its geographical proximity. Relatedness of the Malagasy Cicadellinae fauna is less explored. Young (1986) included the Malagasy fauna in his third monograph comprising Old World Cicadellinae. However, he mentioned the rationale behind the placement of Malagasy fauna along with Old World Cicadellinae as "for facility in use" and pointed out the dissimilarity of Malagasy genera with other Old World genera except for *Tettigoniella blandula* (Signoret), *Acopsis* and *Cofana*. The Madagascar fauna grouped with New World cicadellines and the *Cicadella* group in a phylogenetic study that estimated relationships within the entire subfamily (Takiya 2007, unpublished). This study, however, included only one genus from Madagascar. In the present study, more genera from different parts of Madagascar are included with the addition of sequence data from one more gene (28S ribosomal gene) to test the monophyly of the Malagasy cicadelline fauna, to estimate its relationships to those of other continents and to determine its time of origin based on phylogenetic inference. These results are presented in **Chapter 4**.

Chapter 6 includes a taxonomic revision of the leafhopper subfamily Mileewinae from Madagascar. In Madagascar, this subfamily is represented by one genus, *Ujna*. A single species, *Ujna flavidipes*, was previously known from this region (Evans, 1953). The current study revealed several new species. *Ujna* is closely related to another genus, *Mileewa,* and various authors have interpreted the distinction between these two genera differently. The present study discusses the distinguishing characters of these two genera and includes a key to all known genera of this subfamily and to the previously known and new species of the Malagasy fauna of *Ujna*. Traditionally, male genitalia and external characters have been used to distinguish genera and species in this subfamily while female genitalia characters have not been previously studied. The present study is the first of its kind to explore the utility of female genitalia characters.

Chapter 7 summarizes the present knowledge of systematics and biogeography of leafhoppers in Madagascar. Here I provide insights into plausible biogeographic scenario(s) that led to the origin of the present leafhopper fauna of this tropical island based on inferred phylogeny and divergence time estimates of three independent lineages chosen during this study.

Figures



A B

Figure 1.1. Terrestrial connections of Madagascar during Late Cretaceous : A) 'Southern route' hypothesis (adapted from Rage (1996); B) 'Northern route' hypothesis (modified from Rage (2003) and Krause *et al.* (1997). Abbreviations: AU, Australia; GR, Gunnerus Ridge; Inc, Indochina; KP, Kerguelen Plateau; M, Madagascar; nT, northern Tibet; S, Seychelles Plateau; sT, southern Tibet; SA, South America. Dark grey: terrestrial areas; light grey: presumed terrestrial connections.

Chapter 2

Phylogeny and historical biogeography of leafhopper subfamily Iassinae (Hemiptera: Cicadellidae) with special emphasis on the endemic Madagascar tribe Platyjassini

Introduction

Iassinae is one of the 25 subfamilies of leafhoppers and comprises over 2000 species and 146 genera currently placed in eight tribes (Oman *et al.,* 1990; Dietrich, 2005; Dai *et al.,* 2010). Iassine leafhoppers are mostly arboreal and are distributed worldwide but most of the tribes and genera are restricted to a single biogeographic realm.

Members of this subfamily can be recognized by the following features: head with lateral frontal sutures extended only part way to ocelli, ocelli well separated from eyes and either on the crown or on the anterior margin, forewing with inner apical cell narrow, attenuate, sometimes united with appendix, dorsal margin of female second valvula with a few prominent, widely spaced teeth.

The taxonomic history and distribution of the eight currently recognized tribes (*sensu* Dietrich 2005) are as follows:

Bythonini is a monobasic Neotropical tribe described by Linnavuori (1959). There have been different opinions about whether this tribe should be included in subfamily Iassinae or as a separate subfamily. Evans (1947) placed the type genus *Bythonia* Oman in subfamily Iassinae while Linnavuori (1959) treated this group as separate subfamily (Bythoniinae) and discussed its relationship with Nioniinae. Later Blocker and Webb (1990) placed it in subfamily Iassinae and mentioned its similarity with New World Iassine genera. Dietrich (2005) treated this as one of the eight tribes in subfamily Iassinae. Bythoniini are recorded only from South American rainforests.

The endemic Australian tribe Trocnadini was established by Evans (1947) based on type genus *Trocnada* Walker (1858). These leafhoppers are strictly arboreal and associated with eucalypts. The tribe can be recognized by the crown with a unique declivous shape and a distinct bend at the level of the antennal ledges (Evans, 1947; Linnavuori and Quartau, 1975; Dietrich, 2005, Dai and Dietrich, 2010). Linnavuori and Quartau (1975) suggested that it is closely related to tribe Iassini and Dai and Dietrich (2010) noted its similarity to the iassine genera *Batracomorphus* and *Thallatoscopus*.

Iassini is the largest tribe in Iassinae and is distributed worldwide, with over 570 species and 38 genera. Dietrich (2005) synonymized Old World tribe Hyalojassini Evans (1972) based on the characters of the head and pronotum. Iassini *sensu* Dietrich (2005) is poorly defined at present but most members may be recognized by the following combination of characters: head rounded in profile, without sharp transition between crown and face, ocelli not or partially visible in dorsal aspect, hind wing veins RP and MA confluent, female second valvulae with 2-3 widely spaced dorsal teeth (Dai and Dietrich, 2012). Some New World genera presently placed in Iassini have hindwing veins RP and MA separate. Although most of these leafhoppers are arboreal, some species are found on herbaceous plants (Linnavuori and Quartau, 1975).

Tribe Krisnini has been given different taxonomic status by several authors. Metcalf (1966) treated it as a tribe in Iassinae. Ishihara (1961) upgraded it to an independent family, Krisnidae, while several authors considered it as separate subfamily (Linnavuori and Quartau, 1975, Oman *et al.*, 1990, Dietrich, 2005; Viraktamath, 2006). Members of the tribe can be distinguished from other iassines based on the body size relatively large, forewing venation apically reticulate, and hind wing submarginal vein extended onto the jugal lobe. Krisnini is widely distributed in the Old World, but most speciose in the Oriental region. Some species described from Puerto Rico were considered to be of adventitious origin by Linnavuori and Quartau (1975), but a fossil representative from Dominican amber indicates that the group has inhabited the Greater Antilles for at least 25 million years (Dietrich and Vega, 1995). These leafhoppers have been recorded from plants belonging to the families Euphorbiaceae and Piperaceae (Viraktamath 2006).

Another Australian endemic tribe, Reuplemmelini was established by Evans (1966) based on type genus *Reuplemmeles*. This tribe can be distinguished by the following combination of characters: crown flattened, ocelli on or near margin of crown, hindwing veins RP and MA confluent, and male genital capsule slightly retracted into segment VIII.

Selenomorphini was established by Evans (1974) based on *Selenomorphus* Evans. Species of this tribe are recorded only from New Caledonia and the Loyalty Islands. The tribe is characterized by RP and MA hind wing veins separate, ocelli on the anterior margin of the crown and the forewing without an appendix (Evans 1974).

Tribe Gyponini is widely distributed in the Nearctic and Neotropical regions. Linnavuori and Quartau (1975) followed by Dietrich (1993) placed Gyponini as a tribe of Iassinae whereas

previously many authors treated Gyponinae (=Scarinae) and Iassinae as separate subfamilies. Recent phylogenetic analyses (Dietrich *et al.*, 2001; 2005) suggest that these two family-group taxa are closely related.

Platyjassini is a monobasic tribe endemic to Madagascar erected by Evans (1953) with type genus *Platyjassus* Evans. This tribe can be recognized by the following combination of characters: crown flattened, ocelli on crown distant from margin, hindwing veins RP and MA fused apically. Presently, four species have been described from Madagascar (Evans, 1953; 1959).

Hamilton (1983) suggested that Iassinae (*sensu lato*) is polyphyletic and placed the previously recognized tribes in two separate subfamilies, Jassinae and Scarinae. More recent phylogenetic analyses of the entire family Cicadellidae based on morphological and molecular data (Dietrich, 1999; Dietrich *et al.*, 2001; 2010) recovered Iassinae, in the present sense, as monophyletic. Recently, Dai and Dietrich (2010) estimated phylogenetic relationships among major lineages of Iassinae for the first time using 89 adult morphological characters. Based on this study, all tribes within the subfamily except Iassini were monophyletic. The Madagascar endemic tribe, Platyjassini was placed as sister to endemic New World tribe Gyponini. Moreover, this Malagasy lineage is nested within a clade that includes Krisnini, some New World genera currently placed under tribe Iassini and tribe Selenomorphini and New World Gyponini. The remaining taxa of the subfamily were grouped together with high Bremer support.

The family Iassinae is of biogeographical interest because of its occurrence in fragments of the former Gondwanan continents and the presence of endemic tribes limited to a particular biogeographic realm. Knight and Nielson (2001) suggested the Old World tropics as center of origin for this subfamily based on species richness and distribution. Nevertheless, no detailed studies based on phylogeny have been done to investigate the biogeographical history of the group. Based on their preliminary analysis, Dai and Dietrich (2010) suggested that a combination of continental vicariance and dispersal contributed to the diversification of major iassine lineages. The also suggested that dispersal from Southeast Asia into the New World occurred, based on the placement of a grade of Southeast Asian taxa subtending the clade comprising most New World genera of Iassini sensu Dietrich (2005). Placement of the endemic Malagasy tribe Platyjassini as sister to the endemic New World tribe Gyponini suggests the possibility of continental vicariance in shaping the Malagasy leafhopper distribution. This

vicariant pattern is quite different from the more common pattern of transoceanic dispersal of fauna from Africa and Asian continents reported in the literature (Fisher, 1997; Kerdelhue *et al.*, 1999; Fuller *et al.,* 2005; Monaghan *et al.,* 2005; Wirta *et al*., 2008). However, addition of molecular data is required to test the robustness of the phylogenetic estimate based only on morphology.

My goals in the present study are 1) to reexamine the phylogeny of this subfamily using molecular data and compare its congruence with the morphology-based results; 2) to estimate dates of divergence of major lineages in the subfamily and correlate these with geological events to understand the influence of historical processes on the geographic distribution of extant taxa.

Materials and Methods

Taxon sampling

Fifty-three taxa were included representing all eight tribes recognized to date. Table 2.1 shows the list of taxa and DNA sequences included in phylogenetic analyses. All currently recognized tribes of the subfamily are represented at least by one genus in the dataset. I selected five taxa belonging to three related subfamilies, Hylicinae (*Hatigoria* sp.), Ledrinae (*Xerophloea* sp., *Stenocotis* sp., *Putoniessa* sp.) and Tartessinae (*Tartessus* sp.) as outgroups based on their relatedness to Iassinae (Dietrich *et al.,* 2004). Voucher specimens are deposited in the insect collection of the Illinois Natural History Survey.

Isolation of DNA, amplification, sequencing and alignment

Total genomic DNA was extracted from leafhopper specimens preserved in 95% ethanol except in the case of *Nigrojassus* sp. where DNA was extracted from legs of a dry specimen. Either the abdomen or legs were used for DNA extraction for the remaining taxa depending on the size of the specimen. Later, abdomens were dissected and placed in genitalia vials pinned along with the rest of the body. DNA extraction was performed with a DNeasy Tissue Kit (Qiagen, Valencia, CA). Fragments of the nuclear genes 28S rRNA (28S) and Histone H3 (H3) and the mitochondrial gene 12S rRNA (12S) were amplified using primer pairs indicated in Table 2.2. These genes were selected based on their wide phylogenetic utility in published studies on leafhoppers. A previously published dataset of nearly complete 28S gene sequences for leafhoppers (Dietrich *et al.,* 2001) was screened and the D2, D8 and D9-10 regions were

selected for amplification, based on the presence of many characters informative within this subfamily. 28S rDNA sequences of 11 taxa including outgroups were obtained from Genbank (Dietrich *et al*., 2001) and from an ongoing phylogenetic study of family Cicadellidae by Dietrich *et al.* (unpublished). Histone H3 and 12S sequences were amplified for some of the taxa for which only 28S sequences were available previously using DNA extracted from original voucher specimens listed by Dietrich *et al.,* (2001). Amplified PCR products were purified using Qiaquick PCR purification kit (Qiagen) and were sequenced in both directions using ABI Prism BigDye Terminator Kit version 3 (PE Applied Biosystems, Foster City, CA). Sequencing products were run on an ABI 3730XL capillary sequencer. Chromatograms of both forward and reverse sequences were visualized in Sequencher 4.7 (Gene Codes, Ann Arbor, MI). Aligned contigs were subjected to multiple sequence alignment in Opal (Wheeler and Kececioglu, 2007) installed in Mesquite v2.73 (Maddison and Maddison, 2010), and then edited manually to fix obvious misaligned regions. In order to include information from insertions and deletions (indels) in 28S sequences, gaps were coded using the program Seqstate v.1.32 (Müller, 2004). The modified-complex-indel-coding scheme (MCIC; Müller, 2006) was chosen based on its better performance compared to other indel-coding approaches (Simmons *et al.,* 2007). In their simulation based parsimony studies on different indel coding methods, two methods were suggested as the best: MCIC and SIC (simple indel coding), in which MCIC outperforms SIC in the treatment of overlapping indels. SIC treats these indels as missing data whereas MCIC uses multistate characters to code overlapping indels and assign a distinct symmetrical step matrix to those indels.

Models of DNA sequence evolution were chosen using jModelTest 0.1.1 (Posada, 2008) under the Akaike information criterion. The GTR+I+Γ model was indicated as the best-fit model for all genes, and was further used in all BI analyses.

Phylogenetic analyses

Separate analyses of morphology and DNA sequences and the concatenated dataset of both morphology and DNA were done using maximum parsimony (MP) in PAUP v4.0b10 (Swofford, 2002), maximum likelihood (ML) in GARLI v2.0 (Zwickl, 2006) and Bayesian analysis (BI) with Markov Chain Monte Carlo sampling in MrBayes v3.04 (Ronquist and Huelsenbeck, 2003).

Parsimony analyses were done using a heuristic search with 10,000 random addition sequence replicates and tree-bisection reconnection (TBR) branch swapping. Branch support was estimated using nonparametric bootstrapping (Felsenstein, 1985) using 500 parsimony bootstrap pseudoreplicates with 10 random addition sequence replicates per pseudoreplicate.

Maximum likelihood analyses in GARLI were carried out with twenty independent search replicates, with each replicate run for 1,000,000 generations. Separate data partitions (subsets) were set up for each gene, indel and morphology. The GTR+Gamma model for molecular data partitions and Mk model (Lewis, 2001) for indel and morphology datasets were specified. Values of all other parameters were set to the default options. Consensus trees from the bootstrap output were generated using PAUP. Bootstrap values were estimated by running 100 bootstrap replicates, with five searches per replicate for each of 100,000 generations.

Bayesian analyses were performed with four Markov chains, three heated and one cold, each initiated with a random tree and two independent runs each for 10,000,000 generations, sampling every 100th generation. In Bayesian analysis, four Markov chains, three heated and one cold, were started from a random tree and all four chains ran simultaneously for 10,000,000 generations on four unlinked data partitions based on gene sequence and indels. Separate data partitions were defined and unlinked for the 28S rDNA data, indel dataset, histone H3, 12S and morphology. Trees were sampled every 100th generation. Default options were used for all the parameters of prior distribution. The GTR+I+ Γ model was used for all molecular data partitions. The indel and morphology datasets were run under the standard discrete model. Stationarity of the Markov Chain was determined as the point when sampled log likelihood values plotted against generation time reached a stable mean equilibrium value; the first 20% MCMC samples were discarded as "burn in". Convergence of the Markov Chain and assessment of "burn in" samples were determined using Tracer v1.4 (Rambaut and Drummond, 2003). Branch lengths of the majority rule consensus tree were obtained using the "sumt" option of MrBayes.

Divergence time estimation

Divergence dates were inferred using a Bayesian relaxed clock uncorrelated lognormal method in BEAST (Drummond and Rambaut, 2007) for all data combined with three data partitions for 28S, Histone and 12S. The partitioned BEAST .xml input file (available on request from the corresponding author) was created with BEAUti v1.4.6 (Drummond and Rambaut,

2007). A smaller dataset that includes only taxa with molecular sequences are included in the analysis. A separate GTR+I+Γ model with four gamma categories was selected based on jModelTest (Posada, 2008) and a Yule prior was set for branch lengths. Model parameters were unlinked across partitions. After an initial period of fine-tuning the operators, two separate MCMC analyses were run for 10 million generations with parameters sampled every 1000 generations. Independent runs were combined using LogCombiner1.5.4, and the first 20% of the generations from each run were discarded as "burn in". Convergence of the chains was checked using TRACER v1.4.1 (Rambaut and Drummond, 2003). The searches achieved adequate mixing as assessed by the high effective sampling size (ESS) values for all parameters. Node ages and upper and lower bounds of the 95% highest posterior density (HPD) interval for divergence times were calculated using TreeAnnotator v.1.5.4 (Drummond and Rambaut, 2007) and visualized using FigTree v.1.3.1 (Rambaut, 2010). The 95% HPD represents the shortest interval that contains 95% of the sampled values from the posterior (Drummond *et al.*, 2007). Another analysis was run with same prior settings (Table 2.3) without data to check whether the priors are producing the results.

Calibrations

Due to the paucity of fossils that can be specifically assigned to the Iassinae lineage, I chose the oldest undoubted cicadellid fossil (~118 mya) (Hamilton, 1990; 1992; Shcherbakov, 1992) to constrain the maximum age of the root of the tree. Cretaceous fossil Cicadellidae other than two fossils tentatively assigned to Ledrinae have all been assigned to extinct subfamilies. Leafhoppers belonging to other modern subfamilies do not appear in the fossil record until the Tertiary (Baltic amber). Thus, it is plausible to assume that the origin of Iassinae could not have occurred before the Cretaceous, given the lack of evidence of most modern subfamilies during that time. The normal distribution was used as a prior for this node (A in Fig. 3), with a mean at 118 Ma (std. dev- 6) and 97.5% confidence interval (CI) between 108 and 129.8 mya. A normal distribution was chosen because it allows uncertainty in the calibration estimates (Ho SYW, 2007). The earliest available fossil of *Krisna* (Dietrich and Vega, 1995) from Dominican amber was used to calibrate the split between the tribe Krisnini from rest of the taxa in the tree. The age of Dominican amber, 15-20 mya based on Foraminifera (Iturralde-Vinent and MacPhee, 1996) was used to calibrate this node (C in Fig. 3)(minimum age). A lognormal prior distribution

was specified for this internal calibration (Table 3) that covered a wider time range and allowed the date to shift backwards in time because the divergence event is likely to have occurred prior to the appearance of the earliest fossil (Ho & Philips, 2009). Geologic calibration (node B in Fig. 3) that corresponds with the split between South America and Africa at about 110-100 mya was used to constrain endemic New World tribe Gyponini (maximum age). The last known connection between these two continents is estimated to be around 90 mya (Pitman *et al.*, 1993). A normal prior with mean 105 was used to calibrate this node. Considering errors associated with age calibration (Heads, 2005), three different calibration schemes were used to test sensitivity of calibration priors: 1) included only root age and *Krisna* fossil, leaving out geologic calibration of the Gyponini clade; 2) included all three calibrations, root age (mean: 118) fossil and geologic calibration; 3) set a different root age by assigning a normal prior with lower bound set to 118 mya based on the earliest known leafhopper fossil and upper bound as 217 mya based on the molecular age estimate for the age of angiosperms (Smith *et al*., 2010)

Results

Phylogenetic analyses

The aligned DNA sequence dataset included 2057 positions of 28S including indels, 348 positions of histone and 401 positions of 12S. In the combined dataset of both morphology and molecules, 1942 characters were constant, 591 variable characters were parsimony informative and 253 variable characters were parsimony uninformative.

ML analysis of the combined dataset yielded trees with likelihood scores between -20253 and -20065, with highest score of -20065.1189.

The phylogram from Bayesian analysis of combined data (morphology and molecules) is shown in Fig. 1. Results from separate analyses using different datasets (morphology, molecular dataset, morphology+molecular dataset) and different methods of analyses (MP, ML and Bayesian) were mostly congruent. The tree constructed from the combined dataset including all three loci was better resolved than individual locus trees and had higher branch support values for major clades.

The Bayesian and Maximum Likelihood topologies were more resolved compared to the Maximum Parsimony bootstrap consensus, especially at deeper nodes. All analyses recovered several well-supported monophyletic groups. Monophyletic clades included mostly taxa confined

to a particular biogeographic realm and many of these groupings closely agree with the current tribal groupings based on morphology.

The Neotropical tribe *Bythonini*, represented by genus *Bythonia* consistently grouped among the outgroups in all analyses, but with low branch support. The New World tribe, Gyponini was recovered as a well supported monophyletic clade, placed consistently near the base of the tree, but its relationship with rest of the taxa is uncertain. Another South American endemic genus, *Nigrojassus* was recovered as sister group to Gyponini, but branch support for this relationship is very low. The Malagasy endemic tribe Platyjassini was monophyletic with two well supported clades and was consistently recovered as the sister clade to the rest of the Iassinae tribes (node A) with high support. The remaining taxa from Iassinae fall into one large well supported monophyletic clade (node B) that contains monophyletic Krisnini, a well-supported clade with the Australian tribe Selenomorphini plus two New World genera currently placed in Iassini, and another monophyletic clade that contains Reuplemmelini, Trocnadini, and the remaining genera currently placed in tribe Iassini. A large clade of New World Iassinae was nested within group of genera from Southeast Asia, a pattern similar to that recovered in the morphology-based tree (Dai and Dietrich 2010).

The overall tree topology was largely the same in both independent analyses of morphological and molecular datasets with several monophyletic clades but with less support in relationships among the clades near the base of the tree compared to the tips. Notable discrepancies are: 1) the morphology tree recovered a monophyletic clade (Bremer support value=1) including Platyjassini, Krisnini and Gyponini, Selenomorphini and New World genera (*Pachyopsis* and *Scaroidana*), whereas in the molecular tree, these taxa form a paraphyletic grade; 2) Platyjassini is placed as sister to the rest of the Iassinae tribes in the tree based on DNA sequences alone and the combined data set, but in the morphology tree Platyjassini grouped with New World Gyponini.

Divergence times and biogeography

Estimated ages inferred for nodes of biogeographical interest under different calibration schemes are shown in Table 2.4. The chronogram based on calibration scheme 1 was selected over schemes 2 and 3 in order to avoid possible circularity associated with geologic calibration (Swenson *et al.,* 2012). The divergence time of Gyponini crown age under scheme 2 and 3 was

much older and those of the rest of the lineages were also consistently older (+10 to 40 mya). The results discussed below are based on scheme 1. Fig. 2 shows the chronogram resulting from calibration scheme 1. The age estimates of clades of interest are listed in Table 2.4. The topology from the BEAST analysis is highly congruent with the Bayesian phylogram. Because BEAST arbitrarily resolves polytomies, slight variation (grouping of *Nigrojassus* and *Bythonia* together) occurred between the topology of the chronogram and that of the Bayesian phylogram. Divergence time estimates of these taxa are not taken into account because the relationship is not well resolved.

Discussion

Phylogeny

All phylogenetic analyses recovered a tree with moderate to high branch support for most of the branches near the tip. However, the topology at the base of the tree was not well supported in all analyses and the relationship of Iassinae to other leafhopper lineages remains unclear. Placement of *Bythonia* with the outgroup and presence in this taxon of unique morphological characters quite different from rest of the Iassinae indicate that this genus may not belong to Iassinae. Another ambiguous relationship is that of Neotropical genus, *Nigrojassus*, which is presently unplaced to tribe due to its unique morphological features. In an independent analysis in which this genus is excluded, branch support of the node comprising Gyponini and the rest of Iassinae improved somewhat. Failure to resolve the relationships of *Nigrojassus* may be due to the availability of only partial sequence data for this taxon.

Phylogenetic placement of the Malagasy tribe Platyjassini as sister to the rest of Iassinae (excluding New World tribe Gyponini) and its consistent placement as one of the deeper nodes within Iassinae suggest that this endemic tribe belongs to an ancient lineage within the subfamily that became isolated and diversified within Madagascar. This tribe was recovered as monophyletic with high branch support in all analyses.

Although the main focus of this study was to investigate the relationship of Platyjassini to other tribes, the analysis also revealed some interesting phylogenetic relationship among other taxa. The most notable ones are

1) Grouping of New Caledonia endemic tribe Selenomorphini with New World genera *Pachyopsis* and *Scaroidana* with high support in all analyses

2) The tribe Gyponini is recovered consistently outside the clades of other iassine tribes.

3) Placement of most New World genera of Iassini in a lineage subtended by Oriental genera, including those formerly placed in Hyalojassini, suggests that most New World Iassini are derived from an ancestor that dispersed from the Oriental region. This suggests that tribe Hyalojassini should be reinstated and expanded to include most of the New World genera currently placed in Iassini.

4) *Trocnada* (Trocnadini), endemic to Australia, is closely related to *Thalattoscopus*, an endemic Australian genus currently placed in Iassini.

5) Placement of *Platyhinna* (formerly included in Ledrinae) within the New World clade of Iassini, in agreement with the results of Jones and Deitz (2009).

6) The Palearctic genus *Iassus* belongs to a clade comprising three endemic Afrotropical genera.

Blocker (1979) estimated the phylogeny of New World Iassinae based on a manual, Hennigian-style analysis of morphological characters. Blocker included all the New World genera (excluding Gyponini and Krisnini), but did not consider the possibility that New World Iassinae (sensu stricto) might be polyphyletic. The present results are more or less in agreement with Blocker's phylogeny, although New World Iassinae is recovered as polyphyletic with at least two different lineages. *Pachyopsis* and *Scaroidana* are not closely related to the rest of the New World genera (*Momoria*, *Penestragania*, etc.), which are more derived and grouped with Southeast Asian genera.

Divergence times and biogeography

The divergence time analysis relies on two fossil calibrations, one at the root and one internal calibration with the Dominican amber fossil of *Krisna*. Divergence time estimation suggests rapid diversification among tribes of Iassinae at the deeper nodes (Fig. 2) (Table. 4). Present divergence analyses show diversification of the majority of tribes in this subfamily occurred within a span of 25-30my during the mid to late Cretaceous. Divergence of Node 7 with Australian endemics and members of Old and New World genera belonging to tribe Iassini sensu Dietrich (2005) occurred mainly during the early Tertiary period.

Divergence of members of the New World endemic tribe Gyponini based on available evidence appears to have occurred around 100 mya (HPD: 81.6, 120). This corresponds well

with the break-up of West Gondwana, i.e., separation of Africa and South America at about 110-100 mya (Raven and Axelrod, 1974, Loernce, 1985) and can be hypothesised that the origin of Gyponini coincided with isolation of South America from the other continents.

The split between the Malagasy endemic tribe Platyjassini from the rest of Iassinae is likely to have occurred during the late Cretaceous when Madagascar was joined with the Seychelles-Indian block (100-88 mya). The ancestral stock of this tribe may have become isolated in Madagascar before it separated from the Indian block. The crown age of this endemic tribe is estimated to be 42 mya, which suggests its diversification occurred within the island after initiation of orographic precipitation during the late Paleocene/early Eocene (Yoder and Nowak, 2006).

The crown age of the clade (node 6) containing the New Caledonian tribe Selenomorphini and New World genera *Pachyopsis* and *Scaroidana* suggests its divergence occurred during late Cretaceous (67 mya). Although, New Caledonia separated from Australia during the late Cretaceous with the opening of the Tasman Sea during the Gondwana break up (Head, 2010), geologic evidence indicates the existence of land continuity between New Caledonia and Australia until the Early Cenozoic (65-61 mya) and most of these land fragments did not submerge until the early Eocene. Occurrence of other ancient clades of New Caledonian biota in Australia, resulting from the Cretaceous split has also been reported (Crisci *et al.,* 1991; Duangjai *et al.,* 2009; Ladiges and Cantrill, 2007). Another leafhopper tribe, Macroceratogoniini, is restricted to New Caledonia and northern Australia. Several studies have reported biotic exchange between Australia and South America through Antarctica via the land bridge that existed until the late Cretaceous. The existence of a leafhopper clade comprising New World and New Caledonian endemic genera provides further evidence of a prior land connection between Australia and South America, via Antarctica.

Divergence of tribe Krisnini appears to have occurred around 40 mya. The disjunct distribution of this group in the Oriental and Caribbean regions and the availability of a *Krisna* fossil in Domincan amber indicates a broader paleodistribution of this group. The present phylogenetic analyses consistently placed the Krisnini clade as sister to the clade comprising Nodes 6 and 7 containing the rest of the Old World and New World iassines (although its relationship with node 6 (*Selenomorphus* + two New World Genera) is not clear). The split of

Node 6 is estimated to be around 102 mya. So it is likely that Krisnini originated during the Cretaceous.

Divergence of Australian endemic tribes Reuplemmelini and Trocnadini is estimated to be around 61 mya and 57 mya, respectively based on current evidence. Divergence of Australian (including New Guinea and New Caledonia) lineages of Myrtaceae is dated to late Cretaceous (Sytsma *et al.*, 2004). *Eucalyptus*, a genus in Myrtaceae is known to be host plants of these tribes (Fletcher, 2004). It has been hypothesized that *Eucalyptus* in Australia appeared in the early Tertiary period when there was an increase in aridity after Australia separated from Antartica (Specht and Specht, 2005). Richness in Australian Hemiptera has been associated with the radiation of eucalypt species (Austin *et al.*, 2004). Thus one plausible hypothesis is that these leafhopper tribes originated when their host plants began diversifying in the Australian region.

The cosmopolitan (except New World) genus *Batracomoprhus* is of relatively recent origin between middle Eocene and late Miocene, which is in agreement with the hypothesis of Linnavuori and Quartau (1975).

The well-supported monophyletic clade (node 10) comprising southeast Asian genera and the most speciose group of New World genera is of biogeographical interest mainly due to its disjunction. The divergence time estimate of this clade indicates a dispersal from Asia to the New World during the late Tertiary period. The most likely biogeographic dispersal pattern that can be hypothesized for this distribution is Cenozoic Beringian dispersal from Asia to New World, a pattern supported by flora and fauna (Sanmartin *et al*., 2001; Guo *et al*., 2012).

Taxonomic implications

Phylogentic relationships inferred here have revealed some previously unrecognized relationships that support formal revision of the tribal placements of some genera. The relationship of the New Caledonian tribe Selenomorphini was not clear previously, but the present analysis shows its affinity to some New World genera with which it shares separate RP and MA hindwing veins. Thus the definition of this tribe can be expanded to include the New World genera *Pachyopsis* and *Scaroidana*. Another taxonomic change is in the status of Australian tribe Trocnadini. The phylogenetic placement of this tribe as sister to Iassini genera may provide justification for synonymizing this tribe under Iassini. Evans (1972) erected the tribe Hyalojassini with two monotypic genera from Southeast Asia. Linnavuori and Quartau

(1975) mentioned the possible relatedness of this group with members of tribe Iassini based on morphology. Dietrich (2005) synonymized this tribe with Iassini based on the shape of the head and pronotum. All analyses both based on morphology and molecular data recovered this Asian taxon grouping with a clade of New World Iassinae that Blocker did not place to tribe. Based on current phylogenetic evidence, the tribe Hyalojassini should be reinstated expanding it to comprise genera from Southeast Asia and the New World. Based on the current phylogenetic analyses, the tribe Iassini should be redefined to include a clade of African taxa and the type genus *Iassus*. Australian tribe Reuplemmelini should be retained whereas Trocnadini, another Australian tribe should be expanded to include *Thalattoscopus* and, possibly *Batracomorphus*, which grouped together in morphology-based analysis.

Conclusions

The present results represent the first attempt to use molecular data to estimate phylogenetic relationships among Iassinae tribes. Overall, results suggest that the molecular phylogeny is highly congruent with morphologically confirmed relationships among taxa. Divergence time estimates revealed the signature of both Gondwanan vicariance and dispersal events in the evolutionary history of this leafhopper lineage. In Madagascar, both Gondwanan relicts and Cenozoic dispersal events shaped the present Iassinae fauna.

Disclaimer : None of the nomenclatural changes in this chapter are considered valid under the ICZN

Figures and Tables

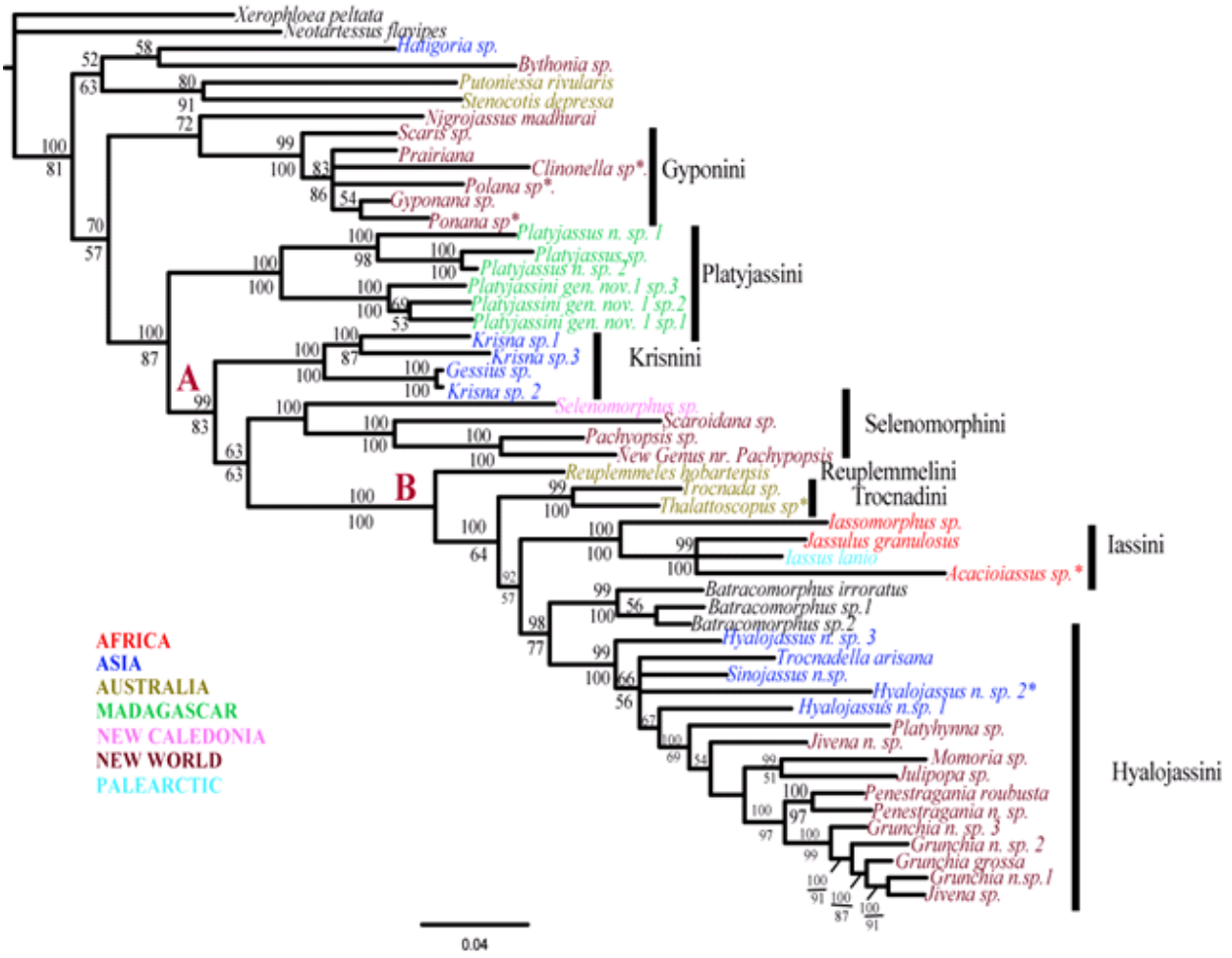


Figure 2.1 Bayesian consensus phylogram of Iassinae. Phylogram from Bayesian analysis of combined dataset of 28S + Indel + Histone + 12S + morphology. Numbers above branches are Bayesian posterior probability (PP) values and the numbers below are bootstrap values from Maximum likelihood analysis. * indicate molecular data of these taxa is not included in the analyses.

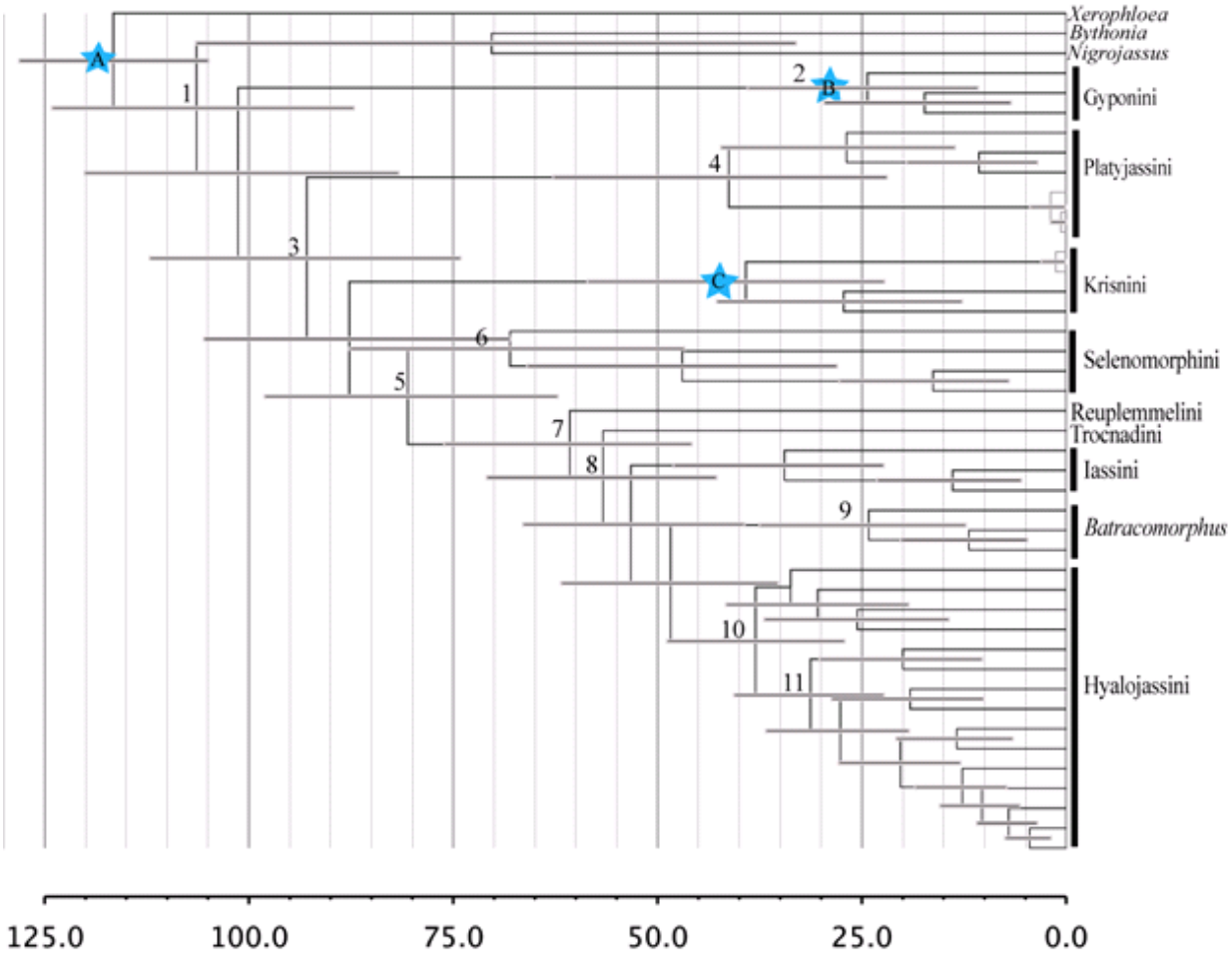


Figure 2.2 Maximum clade credibility tree of Iassinae from BEAST divergence time analysis. Stars with letters indicate calibration points listed in Table 2.3. Numbers correspond to nodes indicated in Table 2.4

Table 2.1 List of taxa and DNA sequences included in the study

Taxa	Voucher ID	Location	Source of 28S sequences*	Sequences obtained for this study		
				28S	Histone	12S
Xerophloea peltata	LH21	North America	AF304617	✔	✔	✔
Neotartessus flavipes	Ah7	Australia	AF304615	✔	✔	✔
Hatigoria sp.	LH168	Asia: Taiwan	AF304663, AF304664	✔	✔	✔
Bythonia sp.	PR49	South America: Ecuador	AF304673	✔	✔	✔
Putoniessa rivularis	Ah3	Australia	Dietrich et al., unpublished	✔	✔	✔
Stenocotis depressa	Ah5	Australia	AF304613	✔	✔	✔
Nigrojassus madhurai	MIAS10	South America: Ecuador	This study	–	✔	–
Scaris sp.	LH175	South America: Peru	Dietrich et al., unpublished	✔	–	–
Gyponana sp.	106	North America	Dietrich et al., unpublished	✔	–	–
Prairiana kansana	LH20	North America	This study	✔	✔	–
Platyjassus sp.	MIAS1	Madagascar	This study	partial sequence	✔	–
Platyjassus n. sp.1	MIAS2	Madagascar	This study	✔	✔	✔
Platyjassus n. sp. 2	MIAS4	Madagascar	This study	✔	✔	✔
Platyjassini gen. nov.1_sp1	MIAS11	Madagascar	This study	partial sequence	✔	✔
Platyjassini gen. nov.1_sp2	MIAS13	Madagascar	This study	partial sequence	✔	✔
Platyjassini gen. nov.1_sp3	MIAS15	Madagascar	This study	partial sequence	✔	✔

Table 2.1 (cont.)

Krisna sp.1	PR56	Asia: Thailand	AF304678	✔	✔	–
Krisna sp. 2	IAS12	Asia: Taiwan	This study	✔	✔	✔
Krisna sp.3	IAS23	Asia: Taiwan	This study	✔	✔	✔
Gessius sp.	IAS11	Asia: Taiwan	This study	✔	✔	✔
Selenomorphus sp.	LH183	New Caledonia	Dietrich et al., unpublished	✔	–	–
Scaroidana sp.	PR50	South America: Ecuador	AF304677	✔	✔	✔
Pachyopsis sp.	IAS1	South America: Peru	This study	✔	✔	✔
New Genus nr. Pachyopsis	IAS3	South America: Peru	This study	✔	✔	✔
Reuplemmeles hobartensis	MIAS14	Australia	This study	✔	✔	✔
Trocnada sp.	LH109	Australia	AF304636	✔	✔	✔
Iassomorphus sp.	IAS17	Africa: Zambia	This study	✔	✔	✔
Jassulus granulosus	IAS16	Africa: Zambia	This study	✔	✔	✔
Iassus lanio	MIAS9	Europe: France	This study	✔	–	✔
Batracomorphus sp. 2	IAS14	Asia: Taiwan	This study	✔	✔	–
Batracomorphus irroratus	Ah2	Asia: Taiwan	AF304583	✔	–	–
Batracomoprhus sp. 1	IAS10	Asia: Taiwan	This study	✔	✔	–
Trocnadella arisana	IAS13	Asia: Taiwan	This study	✔	✔	✔
Sinojassus n. sp.	IAS19	Asia: Thailand	This study	✔	✔	✔
Hyalojassus n. sp. 1	IAS20	Asia: Thailand	This study	✔	✔	✔

Table 2.1 (cont.)

Hyalojassus n. sp. 3	IAS21	Asia: Thailand	This study	✔	✔	✔
Platyhynna sp.	LH179	South America: Peru	Dietrich et al., unpublished	✔	✔	✔
Jivena n. sp.	IAS24	South America: Peru	This study	–	✔	✔
Momoria sp.	59m	South America: Peru	AF304601	✔	–	–
Julipopa sp.	IAS9	South America: Peru	This study	✔	✔	✔
Penestragania robusta	LH63	South America: Peru	AF304627	✔	✔	–
Penestragania n. sp.	IAS2	South America: Peru	This study	✔	✔	✔
Grunchia n. sp. 3	IAS4	South America: Peru	This study	✔	✔	–
Grunchia n. sp. 2	IAS8	South America: Peru	This study	✔	✔	✔
Grunchia grossa	IAS5	South America: Peru	This study	✔	✔	✔
Grunchia n. sp. 1	IAS6	South America: Peru	This study	✔	✔	✔
Jivena sp.	IAS7	South America: Peru	This study	✔	✔	✔

* Numbers indicate GenBank Accession for samples not sequenced for 28S during this study

Table 2.2 Oligonucleotide primers used in this study

Primer	Primer sequence	Source
28SD2F	TGA CCA CGA GAC CGA TAG AA	Modified from Dietrich et al., 2001
28SD2R	TTC GGG TCC CAA CGT GTA CG	Dietrich et al., 2001
28SD8F	CCT CGG ACC TTG AAA ATC C	Dietrich et al., 2001
28SD8R	TGT CTC CTT ACA GTG CCA GA	Dietrich et al., 2001
28SD9F	GTA GCC AAA TGC CTC GTC A	Dietrich et al., 2001
28SD9R	CAC AAT GAT AGG AAG AGC C	Dietrich et al., 2001
HexAF	ATG GCT CGT ACC AAG CAG ACG GC	Ogden &Whiting, 2003
HexAR	ATA TCC TTG GGC ATG ATG GTG AC	Ogden &Whiting, 2003
12Sai	AAA CTA GGA TTA GAT ACC CTA TTA T	Simon et al., 1994
12Sbi	AAG AGC GAC GGG CGA TGT GT	Simon et al., 1994

Table 2.3 Different calibration schemes used in the study. Values represent mean and (in parentheses) 97.5% Confidence Interval

		Scheme 1	Scheme 2	Scheme 3
A	Root	N: 118 (108-129.8)	N: 118 (108-129.8)	N: 167 (118-216)
B	*Krisna* fossil	LogN: 2.995 (18-129.4)	LogN: 2.995 (18-129.4)	LogN: 2.995 (18-129.4)
C	Gyponini clade	---------	N: 105 (100-110)	N: 105 (100-110)

All values are in millions of years before present. N-Normal prior; LogN –logNormal prior

Table 2.4 Estimated node ages (mya) for the nodes labeled in fig. 2.2 under different calibration schemes described in materials and methods and Table 2.3. 95% HPD confidence intervals are given in parentheses.

Node	Crown groups/Splits	Scheme 1	Scheme 2	Scheme 3
1	Gyponini vs. rest	101 (81.6, 120)	119 (110, 129)	171 (131,211)
2	Gyponini crown age	25 (10.7, 38.9)	102 (98, 107)	98 (84, 112)
3	Platyjassini vs. rest	93 (74, 112)	109 (95, 123)	157 (119, 196)
4	Platyjassini crown age	42 (21.9, 62.7)	52 (24, 81)	74.5 (37, 112)
5	Krisnini clade crown age	40 (22, 58.5)	46 (23, 69)	63 (29, 98)
6	Selenomorphini crown age	67 (46.6, 87.6)	75 (51, 100)	112 (71, 153)
7	Reuplemmelini clade+rest	61 (45.8, 76)	75 (59, 90)	105 (76, 135)
8	Iassini stem age	57 (42.7, 70.9)	70 (55, 84)	97 (69,125)
9	Batracomorphus crown age	25 (12, 37.5)	32 (14, 50)	43.5 (19, 68)
10	Hyalojassini crown age	38 (27, 48.8)	47 (33, 62)	67 (44,89)
11	Hyalojassini [Oriental/New World genera split]	31 (22, 40)	36 (22, 50)	54 (35, 73)

Chapter 3

Biogeography of Idiocerine (Hemiptera: Cicadellidae: Idiocerinae) leafhoppers in Madagascar

Introduction

The subfamily Idiocerinae is one of the largest groups of arboreal leafhoppers and is distributed worldwide. These leafhoppers have a narrow, wedge shaped appearance due to their short, broad head. There are over 750 species in 99 genera currently placed in two tribes, Idiocerini and Megipocerini (Zhang & Viraktamath, 2009). In Madagascar, the idiocerine fauna is known from only two published works (Freytag & Knight, 1966; Freytag & Cwikla, 1984), which included 18 described species grouped into 3 genera, *Idiocerus*, *Idioscopus*, and *Nesocerus*. Webb (1983) in his revision of African Idiocerinae, recorded the African genus, *Kopamerra* in Madagascar.

This subfamily was selected for biogeographical study due to following reasons: 1) it is diverse and widely distributed in Madagascar; 2) preliminary examination of Malagasy leafhopper samples revealed many new genera, some of which are endemic.

No prior phylogenetic work has been done on this subfamily. Thus, relatedness of the idiocerine fauna in Madagascar with that of other continents is unclear in spite of its rich diversity and wide distribution. The main objectives of this study are: 1) to test whether the idiocerine fauna in Madagascar is monophyletic; 2) to understand its relationship with the fauna in other continents; 3) to propose a plausible biogeographical scenario that shaped its present distribution based on phylogeny as well as molecular dating analyses.

Materials and Methods

Taxon sampling

Forty-seven taxa were included representing genera from different continents. Table 3.1 shows the list of taxa and DNA sequences included in phylogenetic analyses. Genera belonging to both idiocerine tribes, Megipocerini and Idiocerini are represented in the dataset. I selected seven taxa belonging to four related subfamilies, Agalliinae (*Aceratagallia* sp.), Austragalloidinae (*Austragalloides* sp..), Macropsinae (*Macropsis* sp., *Oncopsis* sp.,

Pediopsoides sp). and Megophthalminae (*Brenda* sp., *Tiaja* sp.) as outgroups based on their relatedness to Idiocerinae (Dietrich et al., 2001). Voucher specimens are deposited in the insect collection of the Illinois Natural History Survey.

Isolation of DNA, amplification, sequencing and alignment

Total genomic DNA was extracted from leafhopper specimens preserved in 95% ethanol. Either the abdomen or legs were used for DNA extraction. Later, abdomens were dissected and placed in genitalia vials pinned along with the rest of the body. DNA extraction was performed with a DNeasy Tissue Kit (Qiagen, Valencia, CA). Fragments of the nuclear genes 28S rRNA (28S) and Histone (H3) were amplified. These genes were selected based on their wide phylogenetic utility in published studies on leafhoppers. A previously published dataset of nearly complete 28S gene sequences for leafhoppers (Dietrich et al., 2001) was screened and the D2, D6 and D8 regions were selected for amplification, based on the presence of many characters informative within this subfamily. Sequences of 28S of outgroups (7 taxa) were obtained from Genbank (Dietrich et al., 2001) and from ongoing phylogenetic study of family Cicadellidae by Dietrich et al. (unpublished). Histone H3 was amplified for taxa for which only 28S sequences were available previously using DNA extracted from original voucher specimens listed by Dietrich et al. (2001). Amplified PCR products were purified using Qiaquick PCR purification kit (Qiagen) and were sequenced in both directions using ABI Prism BigDye Terminator Kit version 3 (PE Applied Biosystems, Foster City, CA). Sequencing products were run on an ABI 3730XL capillary sequencer. Chromatograms of both forward and reverse sequences were visualized in Sequencher 4.7 (Gene Codes, Ann Arbor, MI). Aligned contigs were subjected to multiple sequence alignment in Opal (Wheeler and Kececioglu, 2007) installed in Mesquite v2.73 (Maddison and Maddison, 2010), and then edited manually to fix obvious misaligned regions. In order to include information from insertions and deletions (indels) in 28S sequences, gaps were coded using the program Seqstate v.1.32 (Müller, 2004). The modified-complex-indel-coding scheme (MCIC; Müller, 2006) was chosen based on its outperformance of other indel-coding approaches (Simmons et al., 2007). In their simulation based parsimony studies on different indel coding methods, two methods were suggested as the best: MCIC and SIC (simple indel coding), in which MCIC outperforms SIC in the treatment of overlapping indels. SIC treats

these indels as missing data whereas MCIC uses multistate characters to code overlapping indels and assign a distinct symmetrical step matrix to those indels.

Models of DNA sequence evolution were chosen using jModelTest 0.1.1 (Posada, 2008) under the Akaike information criterion. The General Time Reversible GTR+I+Γ model was indicated as the best-fit model for all genes, and was used in all BI analyses.

Phylogenetic analyses

Separate analyses of morphology and DNA sequences and the concatenated dataset of both morphology and DNA were done using maximum parsimony (MP) in PAUP v4.0b10 (Swofford, 2002), and Bayesian analysis (BI) with Markov Chain Monte Carlo sampling in MrBayes v3.04 (Ronquist and Heulsenbeck, 2003)

Parsimony analyses were done using a heuristic search with 10,000 random replicates and tree-bisection reconnection (TBR) branch swapping. Branch support was estimated using nonparametric bootstrapping (Felsenstein, 1985) using 500 parsimony bootstrap pseudoreplicates with 10 random addition sequence replicates per pseudoreplicate.

In Bayesian analysis, four Markov chains, three heated and one cold, were started from a random tree and all four chains ran simultaneously for 10,000,000 generations on four unlinked data partitions based on gene region and indels. Separate data partitions were defined and unlinked for the 28S rDNA data, indel dataset, and histone H3. Trees were sampled every 100th generation. Default options were used for all the parameters of prior distribution. GTR+I+Γ model was used for all molecular data partitions. The indel datasets were run under the standard discrete model. Stationarity of the Markov Chain was determined as the point when sampled log likelihood values plotted against generation time reached a stable mean equilibrium value; the first 20% MCMC samples were discarded as “burn in”. Convergence of the Markov Chain and assessment of “burn in” samples were determined using Tracer v1.4 (Rambaut and Drummond, 2003). Branch lengths of the majority rule consensus tree were obtained using the "sumt" option of MrBayes.

Divergence time estimation

Methodology used was the same as explained in chapter 2.

Calibrations

The root age prior was given the same settings as in chapter 2. The oldest undoubted cicadellid fossil (~118 mya) (Hamilton, 1990, 1992; Shcherbakov, 1992) was used to constrain the maximum age of the root of the tree. Idiocerine fossils are not so far recorded. The only available fossil close to this subfamily was a Baltic amber fossil (~45 mya) belonging to the related subfamily Macropsinae. This was used as minimum age to calibrate the Macropsinae clade, which is one of the outgroups in this study. A lognormal prior distribution was specified for this calibration (mean: 2.5; SD: 1, offset: 43) that covered a wider time range and allowed the date to shift backwards in time because the divergence event is likely to have occurred prior to the appearance of the earliest fossil (Ho & Philips, 2009).

Results

Phylogeny

All analyses (parsimony and Bayesian approaches) resulted in similar topologies and here I use the phylogram (fig. 3.1) from Bayesian analysis to summarize the results. For phylogenetic reconstruction, the dataset comprised 2496 characters, of which 512 variable characters were parsimony informative and 179 variable characters were parsimony uninformative. All analyses suggest that the Malagasy Idiocerine fauna is polyphyletic, recovered in several clades in the tree either grouping with Asian genera or African genera except one endemic genus, *Nesocerus*.

Clade I, including species of the endemic Malagasy genus *Nesocerus*, is monophyletic with high branch support and it is recovered as sister group to the rest of the idiocerine taxa included in the study. This sister group relationship received good support (posterior probability (PP)=100) in the Bayesian phylogram , but the bootstrap value was lower (76).

Clade II includes two well-supported monophyletic clades (PP=100), one with genera from Australia/ Indo-Australian region and the other clade including South American genera. These two clades were always recovered as sister groups in all the analyses. The third clade, including genera from mainland Africa/Africa+Madagascar, is recovered as sister to clade IV containing two monophyletic clades with moderate branch support (PP=100). The fourth clade has two main clades, one with Asian genera (*Busonia*, *Philipposcopus*) and another with members of the genus *Chunra*. Two Madagascar species were placed within the fourth clade;

one grouped within the clade of Asian genera, and another with *Chunra*/*Chunra*-like genera that has representatives from Asia and Africa.

Clade V comprises two well-supported mononphyletic clades, one containing Holarctic genera (*Rhytidodus* and *Idiocerus*) + one genus from Southeast Asia; and a second including species of the Oriental genus *Idioscopus*.

Another well supported monophyletic clade (clade VI) includes representatives of species from Madagascar of which one species belongs to the genus *Remoya*, previously recorded in Aldabra Island and mainland Africa.

Clade VII includes taxa from Africa, Southeast Asia and Madagascar. Species from Madagascar in this clade were previously placed in the genus *Idioscopus* and these species grouped with the African genera *Pretioscopus*, *Pandacerus*.

Divergence time estimation

The chronogram resulting from BEAST analysis is based on two calibrations, one at the root age and the second at Macropsinae (fig. 3.2). Table 3.2 shows the node age estimates from this analysis. The chronogram recovered in this analysis closely matched the Bayesian phylogram.

Discussion

Phylogeny

The Bayesian phylogram received strong support throughout, both at the base and toward the tips while bootstrap support values were lower among major clades. Based on the results, a majority of Malagasy idiocerine genera (most still undescribed) are related either to Asian or African idiocerines. However, the endemic genus, *Nesocerus* diverged early in the history of Idiocerinae and it does not appear to have close relatives among the taxa from other continents included in the analysis. Morphologically, one of the distinguishing features of the genus *Nesocerus* is the presence of three apical cells bordering the forewing appendix. Although, some Asian taxa (*Busonia* sp.) share this character with the genus, they did not group together in the analysis.

Although the focus of this study was to estimate the phylogenetic relatedness of idiocerines in Madagascar with those of other continents, a few noteworthy findings of non-Malagasy fauna from this study are as below:

1) The South American clade was recovered as sister to the Australian clade and this larger clade is sister to the clade containing most of Asian and African taxa. Genera from South America were listed under the tribe Megipocerini by Isaev (1988) along with a few other genera from Asia and tropical Africa. Isaev (1988) mentioned the possible grouping of Australian endemic genera (eg: *Rosopaella*) as well as Indo-Pacific/Australian genera (e.g., *Balocha*) under this tribe. The present analysis shows these Australian genera are closely related to South American genera. This relationship is also supported by similarity in genitalia characters. Genus *Chunra,* also placed in Megipocerini (Isaev, 1988) grouped with Asian genera belonging to Idiocerini.
2) *Eurymela*, an endemic Australian genus belonging to subfamily Eurymelinae, is recovered in a clade with Australian idiocerine genera.
3) The widespread Holarctic genera *Idiocerus* + *Rhytidodus* are recovered as sister to Oriental *Idioscopus* with strong support.
4) Two Madagascar species presently placed in *Idioscopus* (*Idioscopus hyalinus* Freytag & Knight and *I. oriani* Freytag & Knight in fig.3.1) consistently grouped within a clade including African genera (*Pretioscopus* and *Pandacerus*). This suggests placement of these species in the Oriental genus *Idioscopus* is incorrect. Previous records of *Idiocerus* and *Idioscopus* Madagascar are questionable as these have been based on few specimens and morphological characters. In particular, the distinction between *Idiocerus* and *Idioscopus* from Madagascar has been based only on the number of macrosetae at the hind femur apex. Webb (1983) demonstrated that this character is unreliable. Thus, a revisionary taxonomic treatment of this subfamily in Madagascar is needed.

These results should be interpreted with caution. Although an effort was made to include representative taxa spanning the morphological diversity of Idiocerinae, this study included only a fraction (10%) of the known genera. Thus, all the hypotheses presented here with regard to relationships between major clades should be further investigated through more extensive taxon sampling.

Divergence time estimation

In the chronogram based on two calibrations described above, the split between the Malagasy endemic genus *Nesocerus* and the rest of Idiocerinae is estimated to be around 93 mya and thus it is likely to have occurred during the late Cretaceous when Madagascar was joined with the Seychelles-Indian block (100-88 mya). The ancestral stock of this genus may have become isolated in Madagascar before it separated from the Indian block and later underwent diversification in eastern rainforests.

The rest of the genera from Madagascar included in this study were grouped with Asian or African genera and node ages of these are estimated to be during the Cenozoic Era. This indicates that a majority of the idiocerine lineages present in Madagascar arrived via relatively recent dispersal from Africa and Asia.

Divergence of the Australian clade (including endemic genera, *Rosopaella*, *Eurymela* and Australian-Southeast Asian genus *Balocha*) from the South American clade is estimated to be around 62 mya, based on current evidence. *Eucalyptus*, a genus in Myrtaceae is known to include many host plant species of these endemic Australian genera (Fletcher, 2002). It has been hypothesized that eucalypts in Australia appeared in the early Tertiary period when there was an increase in aridity after Australia separated from Antarctica (Specht and Specht, 2005). Richness in Australian Hemiptera has also been associated with the radiation of eucalypt species (Austin et al., 2004). Thus one plausible hypothesis is that these leafhopper genera originated when their host plants began diversifying in the Australian region.

Figures and Tables

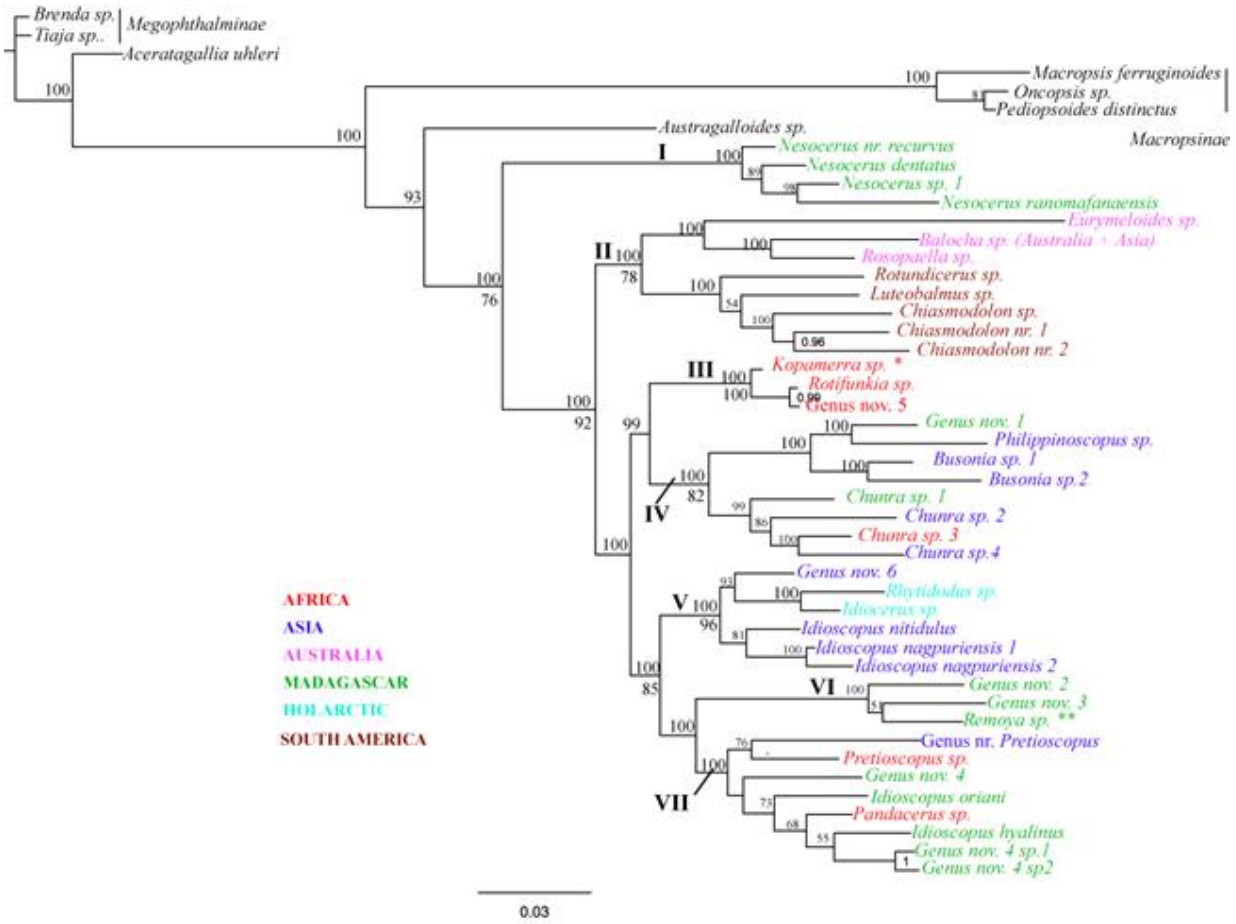


Figure 3.1 Bayesian consensus phylogram of idiocerinae. Phylogram from Bayesian analysis of combined dataset of 28S + Indel + Histone. Numbers above branches are Bayesian posterior probability (PP) values and the numbers below are the bootstrap support values from Maximum parsimony analysis. Symbols * and ** indicate shared distribution with mainland Africa and Aldara, respectively.

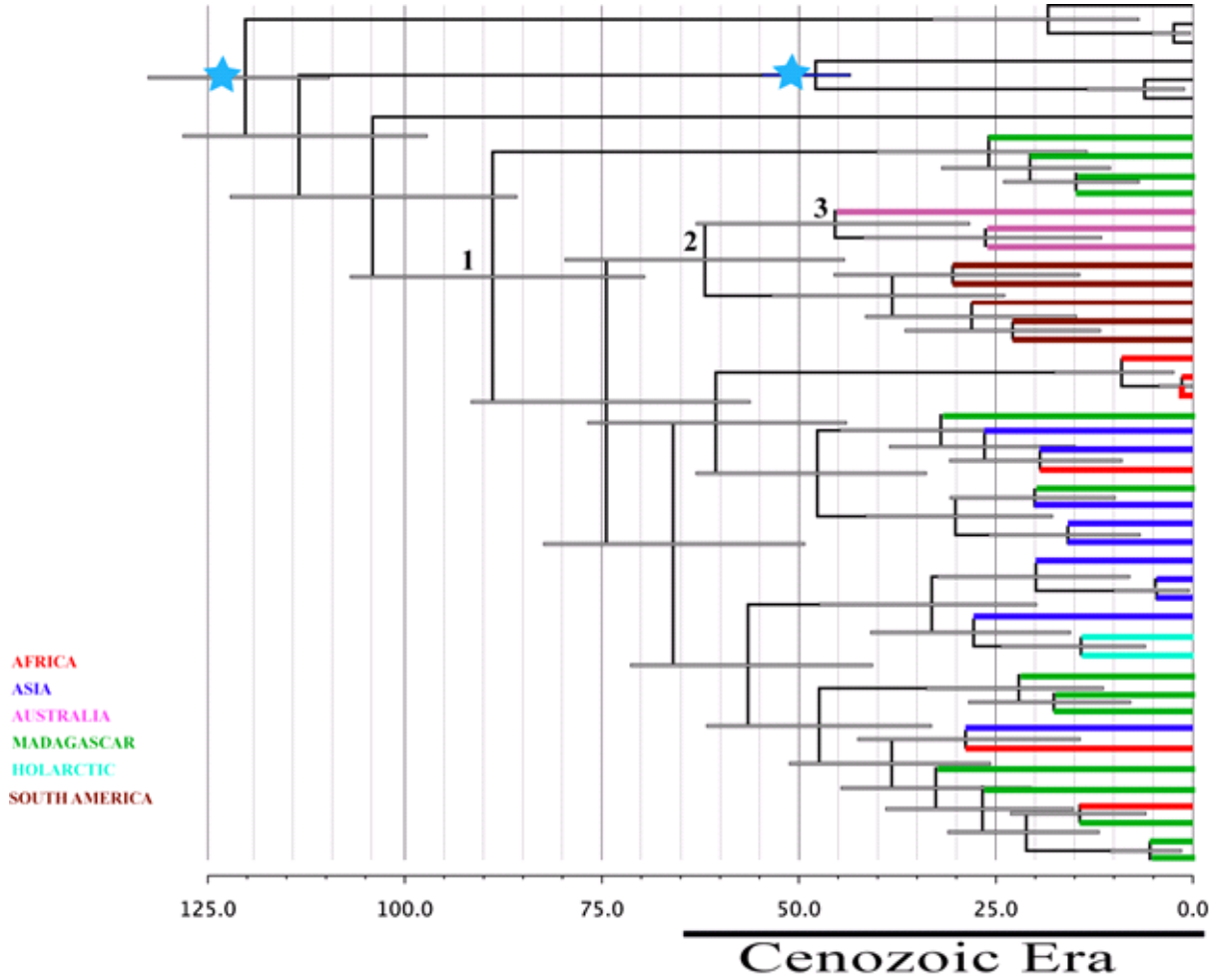


Figure 3.2 Maximum clade credibility tree of idiocerinae from BEAST divergence time analysis. Stars with letters indicate calibration points. Numbers correspond to nodes indicated in Table. 3.2

Table 3.1: List of taxa and DNA sequences of idiocerinae included in the study

Taxa	Voucher ID	Location	Source of 28S sequences*	Sequences obtained	
				28S	Histone
Brenda n. sp.	LH 13	North America	AF304659	✔	✔
Tiaja sp.	LH 55	North America	AF304658	✔	✔
Aceratagallia uhleri	LH 71	North America	AF304645	✔	✔
Macropsis ferruginoides	LH 67	North America	AF304600	✔	✔
Oncopsis sp.	120	North America	Dietrich *et al*., unpublished	✔	–
Pediopsoides distinctus	-	North America	AF304604	✔	–
Austragalloides sp.	LH47	Australia	Dietrich et al., unpublished	–	✔
Nesocerus nr. *Recurvus*	MID32	Madagascar	This study	✔	–
Nesocerus dentatus	CTAB 5	Madagascar	This study	✔	–
Nesocerus sp. 1	CTAB 2	Madagascar	This study	✔	✔
Nesocerus ranomafanaensis	NSID 2	Madagascar	This study	✔	✔
Eurymeloides sp.	MID81	Australia	This study	✔	✔
Balocha sp.	TWID 1	Taiwan	This study	✔	✔
Rosopaella sp.	MID45	Australia	This study	✔	✔
Routndicerus sp.	MID51	South America	This study	✔	✔
Luteobalmus sp.	MID48	South America	This study	✔	✔

Table 3.1 (cont.)

Chiasmodolon sp.	MID46	South America	This study	✔	✔
Chiasmodolon nr. 1	MID47	South America	This study	✔	–
Chiasmodolon nr. 2	MID50	South America	This study	✔	✔
Kopamerra sp.	MID67	Africa	This study	✔	✔
Rotifunkia sp.	MID53	Africa	This study	✔	–
Genus nov. 5	MID78	Africa	This study	✔	✔
Genus nov. 1	MID33	Madagascar	This study	✔	✔
Phillipposcopus sp.	MID54	Asia: Malaysia	This study	✔	✔
Busonia sp. 1	MID64	Asia	This study	✔	✔
Busonia sp. 2	ID8	Asia	This study	✔	–
Chunra sp. 1	MID70	Madagascar	This study	✔	✔
Chunra sp. 2	MID68	Asia: Malaysia	This study	✔	–
Chunra sp. 3	MID52	Africa	This study	✔	✔
Chunra sp. 4	MID55	Asia	This study	✔	✔
Genus nov. 6	MID56	Asia: Malaysia	This study	✔	✔
Idiocerus sp.	MID77	Europe	This study	✔	✔
Rhytidodus sp.	MID80	Central Asia	This study	✔	✔
Idioscopus nagpuriensis	MID63	Asia: India	This study	✔	–
Idioscopus nagpuriensis	ID1	Asia: India	This study	✔	✔
Idioscopus nitidulus	MID65	Asia: India	This study	✔	–
Genus nov. 2	MID31	Madagascar	This study	–	–
Genus nov. 3	MID22	Madagascar	This study	✔	–

Table 3.1 (cont.)

Remoya sp.	MID27	Madagascar	This study	✔	✔
Genus nov. 4	MID60	Madagascar	This study	✔	–
Genus nr. *Pretioscopus*	MID57	Asia: Malaysia	This study	✔	✔
Pretioscopus sp.	MID79	Africa	This study	✔	✔
Idioscopus oriani	MID44	Madagascar	This study	✔	–
Pandacerus sp.	MID41	Africa	This study	✔	✔
Idioscopus hyalinus	MID43	Madagascar	This study	✔	✔
Genus nov. 4_1	MID49	Madagascar	This study	✔	✔
Genus nov. 4_2	MID62	Madagascar	This study	✔	✔

* Numbers indicate GenBank Accession for samples not sequenced for 28S during this study

Table. 3.2 Estimated node ages (mya) for the nodes labeled in fig. 3.2. 95% HPD confidence intervals are given in parentheses.

Node	Crown groups/Splits	Node ages
1	Nesocerus vs rest	88 (69, 106)
2	Australian-SouthAmerican Idiocerinae crown age	61.5 (44, 79)
3	Australian clade crown age	45 (28, 62)

Chapter 4

Phylogeny and historical biogeography of Cicadellinae (Hemiptera: Cicadellidae) in Madagascar

Introduction

Cicadellinae is a cosmopolitan leafhopper subfamily in the family Cicadellidae that comprises over 2000 species in 340 genera (Takiya, 2007). Young (1968, 1977, 1986) in his three monumental monographs placed Cicadellinae in two tribes, Proconiini and Cicadellini. The tribe Proconiini is restricted to the New World, while Cicadellini is distributed both in the New World and Old World tropics. Young (1986) recognized 51 species in 24 genera from Madagascar, of which 21 genera are endemic and 13 are monotypic, and included the Malagasy fauna in his third monograph comprising Old World Cicadellinae. However, he mentioned the rationale behind the placement of Malagasy fauna along with Old World Cicadellinae as "for facility in use" and pointed out the dissimilarity of Malagasy genera with other Old World genera except for *Tettigoniella blandula* (Signoret), *Acopsis* and *Cofana*. These xylem-feeding leafhoppers are brightly colored and are widely distributed in Madagascar, with maximum diversity in humid rainforests.

Young treated New World Cicadellini in various generic groupings, but this was purely based on morphological resemblance without taking into account phylogenetic information. The first phylogenetic attempt to investigate relationships in the subfamily was that of Takiya (2007). In this analysis using both morphology and molecular data, tribe Cicadellini was found to be polyphyletic, with most genera from the Old World and New Guinea grouped together. The two included representatives of the Madagascar fauna grouped with the *Cicadella* generic group, which is restricted to the New World except for one Palearctic genus (*Cicadella*) and one widespread Paleotropical genus (*Cofana*).

Madagascar is well known for its unusual degree (~90%) of endemic plant and animal groups. Origin of the endemic clades has been of great interest to biogeographers due to the complex mix of vicariant and dispersal patterns exhibited by various taxa. The biogeographical origin of Cicadellinae in Madagascar is intriguing for several reasons. This subfamily is species rich and highly endemic in Madagascar. In continental Africa, the group is less diverse (~ seven genera, ~10 species) even compared to rest of the Old World and despite the geographical

proximity of Madagascar to the African mainland. Also, a previous phylogenetic analysis of the entire subfamily suggested that the Malagasy fauna has affinities to New World taxa (Takiya, 2007).

The main objectives of this study are to test the monophyly of cicadellinae in Madagascar and to test two plausible biogeographical scenarios for explaining the origin of the Malagasy fauna based on its grouping with New World cicadellines and the placement of other Old World Cicadellini at the base of the tree in a previous phylogenetic study (Takiya , 2007).

Scenario 1: The ancestral stock of the Madagascar Cicadellinae was present in Indo-Madagascar (~100-80 mya) and became isolated in Madagascar when the Indian block separated from Madagascar. Under this scenario, the relationship between Madagascar cicadellines and the New World fauna may be explained by the exchange of fauna through a terrestrial route between Madagascar and Asia via India and the Seychelles Plateau that existed at the very end of the Cretaceous (~70 mya) ('Northern route' hypothesis by Rage, 1996) and from Laurasia to the New World through North Atlantic connections that existed during the Eocene.

Scenario 2. Exchange of fauna between Indo-Madagascar and South America occurred through a land connection that existed until the Late Cretaceous. Krause et al. (1997) proposed a 'Southern route' i.e. existence of sub aerial contacts between Antarctica and South America in the west and between Antarctica and Indo-Madagascar in the east (Kerguelen Plateau and Gunnerus Ridge) that allowed exchange of biota between these continents.

Scenario 3: Based on the relatively high genus and species diversity of Cicadellinae in South America, the lineage that gave rise to the Malagasy fauna arose in South America. Under this scenario, the ancestral stock of the Malagasy fauna might have dispersed from South America to North America and then, through North Atlantic connections that existed during the Tertiary period, dispersed into Africa and the rest of the Old World. The ancestral stock of the Malagasy lineage might have dispersed from Africa and subsequent African extinction of this group during late Eocene could explain the lower present-day cicadelline diversity in Africa. Thus Madagascar Cicadellinae might

have originated well after the last known connection between Indo-Madagascar and South America.

Materials and Methods

Taxon sampling

Seventy-one taxa were included, with taxa representing 10 genera from Madagascar, 12 taxa representing 7 genera from the rest of the Old World and 29 genera from the New World including both Proconiini and Cicadellini. *Tettigoniella* and *Cofana* are the only genera shared between Madagascar and other continents that were included in this study. Table 1 shows the list of taxa and DNA sequences included in phylogenetic analyses. *Hylaius oregonensis*, *Pagaronia* and *Mileewa* were included as outgroups based on Takiya (2007). Voucher specimens are deposited in the insect collection of the Illinois Natural History Survey.

Isolation of DNA, amplification, sequencing and alignment

Total genomic DNA was extracted from leafhopper specimens preserved in 95% ethanol. To confirm species identifications, abdomens were dissected and placed in genitalia vials pinned along with the rest of the body. Leafhoppers used in DNA extraction were mainly from materials obtained from Jason Cryan (New York State Museum) and from the Madagascar Arthropod project at the California Academy of Sciences. Genomic DNA was extracted using DNeasy Tissue Kits (Qiagen, Valencia, CA). Fragments of the nuclear genes 28S rRNA D8 region (28S) and Histone H3 (H3) and the mitochondrial gene Cytochrome Oxidase II (COII) were amplified. Sequences of all the three genes for Madagascar taxa and 28S sequences of all taxa were newly obtained during this study. Histone and COII sequences of a majority of the New World and some of the Old World taxa used in the study were taken from Takiya (2007) (Table 1). Other parts of the 28S rDNA gene besides the D8 region were found to be uninformative among cicadelline genera. Amplified PCR products were purified using Qiaquick PCR purification kits (Qiagen) and were sequenced in both directions using ABI Prism BigDye Terminator Kit version 3 (PE Applied Biosystems, Foster City, CA). Sequencing products were run on an ABI 3730XL capillary sequencer. Chromatograms of both forward and reverse sequences were visualized in Sequencher 4.7 (Gene Codes, Ann Arbor, MI). Aligned contigs were subjected to multiple sequence alignment in Opal (Wheeler and Kececioglu, 2007) included

in Mesquite v2.73 (Maddison and Maddison, 2010), and then edited manually to fix obvious misaligned regions. In order to include information from insertions and deletions (indels) in 28S sequences, gaps were coded using the program Seqstate v.1.32 (Müller, 2004). The modified-complex-indel-coding scheme (MCIC; Müller, 2006) was chosen based on its outperformance of 10 other indel-coding approaches (Simmons et al., 2007). In their simulation-based parsimony studies on different indel coding methods, two methods were suggested as the best: MCIC and SIC (simple indel coding), in which MCIC outperforms SIC in the treatment of overlapping indels. SIC treats these indels as missing data whereas MCIC uses multistate characters to code overlapping indels and assign a distinct symmetrical step matrix to those indels.

Models of DNA sequence evolution were chosen using jModelTest 0.1.1 (Posada, 2008) under the Akaike information criterion. The General Time Reversible GTR+I+Γ model was indicated as the best-fit model for all genes, and was further used in all BI analyses.

Phylogenetic analyses

Analyses were done using maximum likelihood (ML) in GARLI v2.0 (Zwickl, 2006) and Bayesian analysis (BI) with Markov Chain Monte Carlo sampling in MrBayes v3.04 (Ronquist and Heulsenbeck, 2003). Both combined analysis of all three loci and separate analyses of individual locus were done.

Maximum likelihood analyses in GARLI were carried out with twenty independent search replicates, with each replicate run for 1,000,000 generations. Separate data partitions (subsets) were set up for each gene, and indels. The GTR+Gamma model for molecular data partitions and Mk model (Lewis, 2001) for indel datasets were specified. All other parameters were set to the default options. Consensus trees from the bootstrap output were generated using PAUP. Bootstrap values were estimated by running 100 bootstrap replicates, with five searches per replicate for each of 100,000 generations.

In Bayesian analysis, four Markov chains, three heated and one cold, were started from a random tree and all four chains ran simultaneously for 10,000,000 generations on four unlinked data partitions based on gene sequence and indels. Separate data partitions were defined and unlinked for the H3, COII and 28S and indel dataset. Trees were sampled every 100th generation. Default options were used for all the parameters of prior distribution. GTR+I+Γ model was used for all molecular data partitions. The indel dataset were run under the standard

discrete model. Stationarity of the Markov Chain was determined as the point when sampled log likelihood values plotted against generation time reached a stable mean equilibrium value; the first 20% of MCMC samples were discarded as "burn in". Convergence of the Markov Chain and assessment of "burn in" samples were determined using Tracer v1.4 (Rambaut and Drummond, 2005). Branch lengths of the majority rule consensus tree were obtained using the "sumt" option of MrBayes.

Divergence time estimation

Divergence dates of branching points on the phylogeny were inferred using a Bayesian relaxed clock uncorrelated lognormal method in BEAST (Drummond and Rambaut, 2007) for all data combined with three data partitions for 28S, Histone and COII. The partitioned BEAST .xml input file (available on request from the corresponding author) was created with BEAUti v1.4.6 (Drummond and Rambaut, 2007). A smaller dataset that includes only taxa with molecular sequences are included in the analysis. A separate GTR+I+G model with four gamma categories was selected based on jModelTest (Posada, 2008) and a Yule prior was set for branch lengths. Model parameters are unlinked across partitions. After an initial period of fine-tuning the operators, two separate MCMC analyses were run for 10 million generations with parameters sampled every 1000 generations. Independent runs were combined using LogCombiner1.5.4 (Drummond and Rambaut, 2007), and the first 20% of the generations from each run were discarded as "burn in". Convergence of the chains was checked using TRACER v1.4.1 (Rambaut and Drummond, 2003). The searches achieved adequate mixing as assessed by the high effective sampling size (ESS) values for all parameters. Node ages and upper and lower bounds of the 95% highest posterior density (HPD) interval for divergence times was calculated using TreeAnnotator v.1.5.4 (Drummond and Rambaut, 2007) and visualized using FigTree v.1.3.1 (Rambaut, 2010). The 95% HPD represents the shortest interval that contains 95% of the sampled values from the posterior (Drummond et al., 2007). Another analysis was run with same prior settings without data to check whether the priors are producing the results.

Calibrations

The root node calibration used is same as in chapter 2. I chose the oldest undoubted cicadellid fossil (~118 mya) (Hamilton, 1990, 1992; Shcherbakov, 1992) to constrain the

maximum age of the root of the tree. The normal distribution was used as a prior for this node (A in Fig. 4.3), with a mean at 118 Ma (std. dev- 6) and 97.5% confidence interval (CI) between 108 and 129.8 mya. A second, internal calibration point at the base of the New World Cicadellini clade was done using the earliest available fossil of *Graphocephala* (Dietrich and Vega, 1995) from Dominican amber (minimum age). A lognormal prior distribution was specified for this internal calibration (mean: 2.995; SD-0.89, offset -15) that covered a wider time range and allowed the date to shift backwards in time because the divergence event is likely to have occurred prior to the appearance of the earliest fossil (Ho & Philips, 2009). In a different analysis, I set a different root age (scheme 2) by assigning a normal prior with lower bound set to 118 mya based on the earliest known leafhopper fossil and upper bound as 217 mya based on the molecular age estimate for the age of angiosperms (Smith et al. 2010).

Results

Phylogenetic analyses

Topologies recovered in MP, ML and Bayesian analysis were congruent overall. Some differences were found near the tips. Relationships among major groupings received high to moderate branch support. The tree constructed from the combined dataset including all three loci was better resolved than those based on individual loci and had higher branch support values for major clades.

The dataset including all the three genes comprised 830 nucleotide positions of D8 region of 28S including indels, 350 positions of H3 and 590 of COII. In the combined dataset of all three genes and indels, 1060 characters were constant, 501 variable characters were parsimony informative and 187 variable characters were parsimony uninformative. The aligned dataset of 28S had long insertions (fig. 4.1) shared between New World genera and most of the genera from Madagascar. This long insertion in the D8 region has not, so far, been found in any other leafhopper groups.

In all analyses, Malagasy Cicadellinae are not recovered as monophyletic. They were grouped into two main clades; group 1 and group 2 (fig. 4.2). Madagascar group 1 included one genus (*Madicola*), while rest of the genera were placed in group 2. The genus *Madicola* is so far recorded only from northeastern rainforest (Marojejy National Park) in Madagascar.

The genus *Tettigoniella* has 6 species in central and southern Africa and one species, *T. blandula* in Madagascar. The present dataset included one species from Cameroon and *T. blandula* from Madagascar and these two grouped together and were placed in group 2 in all analyses with high support.

Group 1 is placed as sister to group 2 + New World Cicadellini + *Cicadella* generic group with high branch support (Posterior probability (PP)= 1.0). In Bayesian analysis, these two groups were recovered as two monophyletic clades with high to moderate branch support [PP=1.0 (group 1); PP= 0.89 (group 2)]. Genera belonging to the *Cicadella* generic group were sister to the rest of the New World genera. New World cicadellines were recovered as monophyletic with high branch support in all analyses.

In ML analysis, genera from Asia, Africa and New Guinea were recovered as a single monophyletic clade with good support, but in the Bayesian analysis, the Old World genera formed a paraphyletic grade that gave rise to the Madagascar + New World lineage.

Divergence estimation

Table 2 presents divergence time estimates of for the nodes of interest. Time estimates based on scheme 2 are older (~20 mya) compared to those of scheme 1. Figure 4.3 shows the chronogram based on scheme 1. Scheme 1 was preferred because, based on fossil evidence, modern cicadellid subfamilies were not present during the Cretaceous and the oldest undoubted cicadellid fossil is ~118 mya. While setting the root calibration in scheme 1, uncertainty in calibration estimates was taken into account by assigning a normal prior with 97.5% confidence interval (CI) between 108 and 129.8 mya. In scheme 2, an upper bound was set to 217 mya based on the age of angiosperms that is much older than the oldest known Cicadellidae and this accounts for the older divergence time estimates in that analysis.

Discussion

Phylogeny

Paraphyly of the Malagasy cicadelline fauna is well supported in all analyses. Although group 1 in the present analysis has only one genus, *Madicola*, I assume there may be more genera (not included in the present analysis) in this group. For instance, genus *Malagasicola*

shares both distribution and similarity in genitalia characters with *Madicola*. Group 1 is similar to other Old World Cicadellini in lacking the insertion in the 28S D8 shared by other Malagasy cicadellines with most New World Cicadellini. Monophyly of group 2 was not supported in all analyses. This group was consistently placed either as a monophyletic sister to the clade containing the *Cicadella* generic group and New World endemics or as a paraphyletic grade leading to the *Cicadella* group + New World. This is consistent with the 'Out of Madagascar' dispersal to Laurasia and then to New World scenario.

Divergence estimation

All assumptions stated below are based on two calibrations (root and one internal calibration) available during this study.

The present analysis shows that splits at the deeper levels of this leafhopper tribe occurred during Cretaceous. The crown age of the clades confined to particular geographical regions are estimated to be more recent, i.e., during the Tertiary. Divergence time results of the present study are consistent with biogeographical scenario 1 and/or 2 hypothesized for the origin of the Malagasy fauna.

The split between Old World Cicadellinae and the rest of the fauna (Madagascar +*Cicadella*+New World endemics) (node 1 in fig. 4.3) is estimated to be around 100 mya (This timing coincides with the period of existence of the Indo-Madagascar block. Timing of separation of India from Madagascar is estimated to have started around 88 mya (Simmonds et al. 2012). The crown age of the clade including the Malagasy fauna +*Cicadella*+ New World cicadellini (node 3) is estimated to be ~ 85 mya. This indicates that the divergence of this clade happened after the separation of India from Madagascar. Although this group was not recovered as monophyletic in the Bayesian analysis, the crown age of group 2 of Malagasy fauna is estimated to be around 59 mya (early Eocene) in divergence time analysis. All these genera are distributed in rainforests. This suggests that the radiation within Madagascar occurred after emergence of rainforests during late Paleocene/early Eocene.

Divergence time of the crown age of the clade including the *Cicadella* generic group and New World endemics (node 7) is estimated to be ~70.5 mya (end of Createaceous). This closely agrees with the Northern Route hypothesis, i.e., the terrestrial route between Asia and Madagascar via India-Seychelles plateau that existed at the very end of the Cretaceous.

However, given the 95% confidence interval at node 7, the Southern route hypothesis (land bridge connection, Kergulean plateau (KP) and Gunnerus Bridge between Indo-Madagascar and South America) cannot be ruled out based on the present results. Nevertheless, the Northern Route seems more likely for two reasons. First, the mean crown age of node 7 is estimated to be around 70 mya, well after the submergence of KP (84 mya) (Simmonds et al., 2012). Second, in the present analyses, the *Cicadella* generic group is placed either basal or sister to the New World clade, which suggests a possible route from Laurasia (where *Cicadella* is widely distributed) to the New World through North Atlantic connections (Beringian route) that existed during the Late Paleocene/early Eocene (Townsend et al, 2011). Given the estimated time of origin of New World Cicadellini (61.5 mya), this is a more plausible scenario.

Gondwanan vicariance played major role in the origin of Malagasy Cicadellinae. Within Madagascar, this group later underwent extensive diversification in rainforests.

Figures and Tables

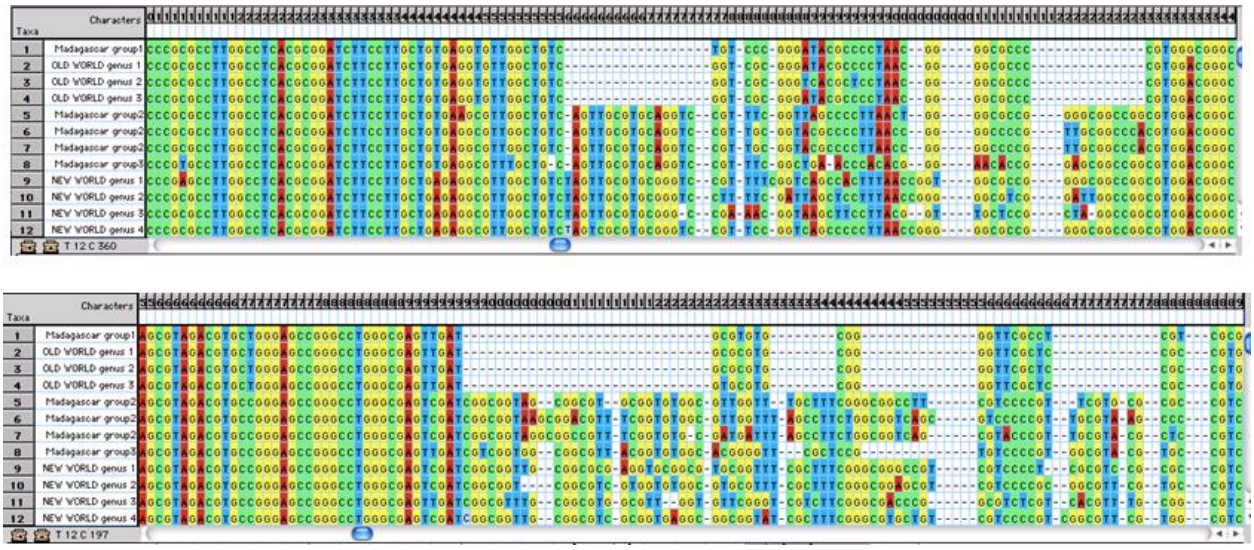


Figure 4. 1 Aligned sequences of D8 region of 28S rRNA showing long insertions of Malagasy group 2 and New World taxa compared to Old World taxa.

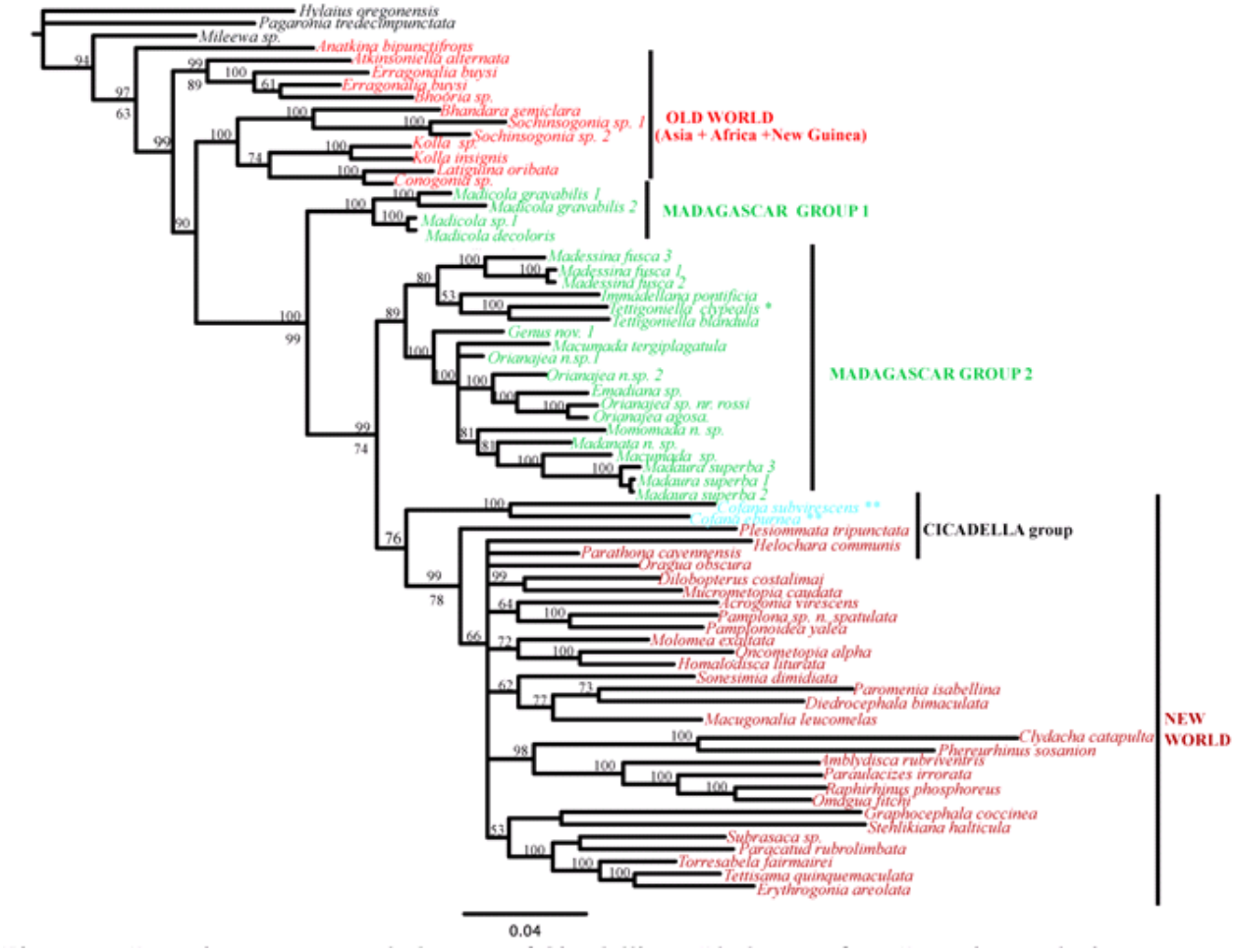


Figure 4.2. Bayesian consensus phylogram of Cicadellinae. Phylogram from Bayesian analysis of combined dataset of 28S +Indel + Histone + COII. Numbers above branches are Bayesian posterior probability (PP) values and the numbers below are bootstrap support values from maximum likelihood analysis. * indicates shared distribution in mainland Africa; ** indicates paleotropical distribution.

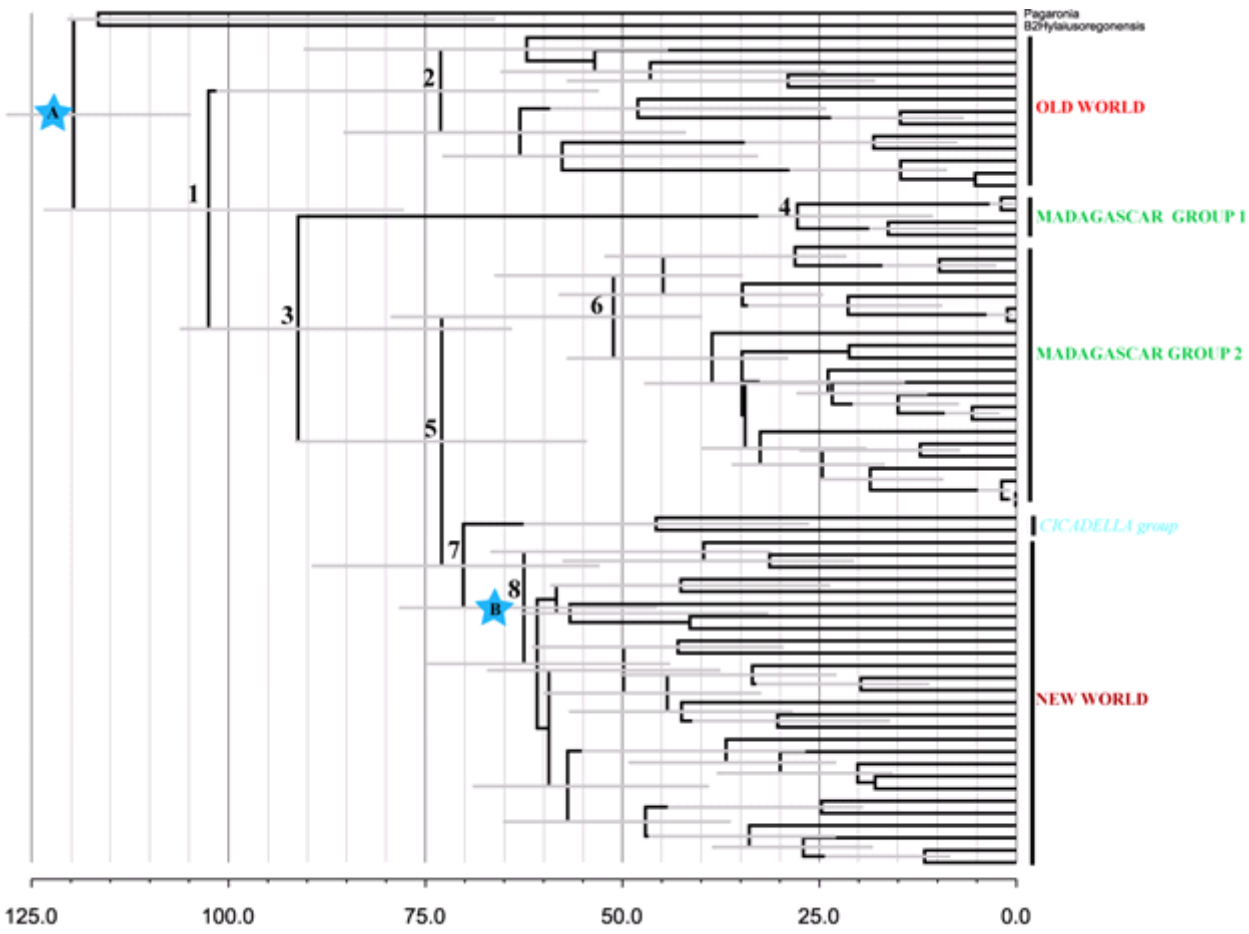


Figure 4.3. Maximum clade credibility tree of cicadellinae from BEAST divergence time analysis. Stars with letters indicate calibration points. Numbers correspond to nodes indicated in Table 4.2

Table 4.1: List of taxa and DNA sequences of Cicadellinae included in the study

Taxa	Voucher ID	Location	Sequences obtained		
			28S	COII	Histone
Hylaius oregonensis	B2	USA	✔	✔*	✔*
Helochara communis	B13	USA	-	✔	✔*
Paromenia isabellina	B47	Costa Rica	-	✔*	✔*
Pagaronia tredecimpunctata	LH 57	USA	✔	✔*	✔*
Homalodisca liturata	LH 97	USA	-	✔*	✔*
Paraulacizes irrorata	LH126	USA	✔	✔*	✔*
Graphocephala coccinea	LH127	USA	-	✔*	✔*
Plesiommata tripunctata	LH128	USA	-	✔*	✔*
Madicola gravabilis	MACI_1	Madagascar	✔	✔	✔
Tettigoniella clypealis	MACI_18	Africa	✔	✔	-
Madicola sp. 1	MACI_2	Madagascar	-	✔	✔
Immadellana pontificia	MACI_24	Madagascar	✔	✔	✔
Macumada sp.	MACI_25	Madagascar	✔	✔	✔
Tettigoniella blandula	MACI_26	Madagascar	✔	✔	✔
Madessina fusca	MACI_27	Madagascar	✔	✔	✔
Macumada tergiplagatula	MACI_28	Madagascar	✔	✔	✔
Madaura superba 2	MACI_29	Madagascar	✔	✔	✔
Madessina fusca	MACI_3	Madagascar	✔	✔	✔
Emadiana sp.	MACI_30	Madagascar	✔	✔*	✔*
Orianajea sp.near rossi	MACI_31	Madagascar	✔	✔	✔
*Orianajea n.sp.*1	MACI_32	Madagascar	✔	✔	✔

Table 4.1 (cont.)

*Sochinsogonia sp.*1	MACI_33	Philippines	✔	✔	✔
Atkinsoniella alternata	MACI_34	Thailand	✔	✔	✔
Kolla sp.	MACI_35	Thailand	✔	✔	✔
Anatkina bipunctifrons	MACI_36	Thailand	✔	✔	✔
Cicadellini *Genus nov.*1	MACI_37	Madagascar	✔	✔	✔
*Erragonalia sp.*1	MACI_38	Thailand	✔	✔	✔
Erragonalia buysi	MACI_39	Thailand	✔	✔	-
Cofana subvirescens	MACI_40	Philippines	✔	✔	✔
Cofana eburnea	MACI_41	Philippines	✔	✔	✔
*Sochinsogonia sp.*2	MACI_42	Philippines	✔	✔	✔
Madicola decoloris	MACI_44	Madagascar	✔	✔	-
*Monomada n.sp.*1	MACI_45	Madagascar	✔	✔	-
Madessina fusca	MACI_46	Madagascar	✔	✔	✔
Madicola gravabilis	MACI_47	Madagascar	✔	✔	✔
*Orianajea n.sp.*2	MACI_48	Madagascar	✔	✔	✔
Orianajea agosa	MACI_49	Madagascar	✔	✔	✔
Madanata n.sp.	MACI_51	Madagascar	✔	✔	✔
Madaura superba 1	MACI_6	Madagascar	✔	✔	✔
Mileewa sp.	LH172	Taiwan	✔	-	-
Madaura superba 3	PR100	Madagascar	✔	✔	✔
Bhandara semiclara	PR103	Thailand	✔	✔*	✔
Amblydisca rubriventris	PR109	Mexico	-	✔*	✔
Dilobopterus costalimai	PR12	Brazil	✔	✔	✔*
Clydacha catapulta	PR125	Peru	-	✔*	✔*
Pamplona sp n. spatulata	PR126	Colombia	✔	✔*	✔*

Table 4.1 (cont.)

Phereurhinus sosanion	PR129	Peru	-	✔*	✔
Acrogonia virescens	PR133	Brazil	-	✔*	✔*
Mucrometopia caudata	PR136	Brazil	✔	✔*	✔*
Molomea exaltata	PR144	Brazil	✔	✔*	✔*
Tettisama quinquemaculata	PR16	Brazil	✔	✔*	✔
Sonesimia dimidiata	PR17	Brazil	✔	✔*	✔*
Latiguina oribata	PR177	New Guinea	✔	✔*	✔*
Torresabela fairmairei	PR19	Brazil	✔	✔*	✔*
Stehlikiana halticula	PR190	Peru	-	✔	✔
Paracatua rubrolimbata	PR192	Colombia	✔	✔	✔
Parathona cayennensis	PR195	Venezuela	✔	✔	✔
Conogonia sp.	PR200	New Guinea	✔	-	✔
Bhooria sp.	PR203	Africa	✔	✔	✔
Oncometopia alpha	PR27	USA	✔	✔*	✔*
Raphirhinus phosphoreus	PR32	Bolivia	✔	✔*	✔*
Pamplonoidea yalea	PR42	Brazil	✔	✔*	✔
Omagua fitchi	PR44	Ecuador	✔	✔*	✔*
Subrasaca sp.	PR68	Brazil	✔	✔*	✔*
Diedrocephala bimaculata	PR77	Brazil	✔	✔*	✔
Oragua obscura	PR78	Mexico	✔	✔*	✔*
Erythrogonia areolata	PR79	Mexico	✔	✔*	-
Macugonalia leucomelas	PR81	Brazil	✔	✔*	✔
Kolla insignis	PR96	Taiwan	✔	✔*	✔*

Table. 4.2 Estimated node ages (mya) for the nodes labeled in fig. 4.3 under scheme 1 described in materials and methods. 95% HPD confidence intervals are given in parentheses

Node	Crown groups/Splits	Scheme 1	Scheme 2
1	Old World Cicadellinae vs rest	100 (77, 123)	120 (68, 173)
2	Old World Cicadellinae crown age	77 (53, 101)	91 (47, 135)
3	Madagascar group 1 vs rest	85 (64, 106)	102 (57, 147)
4	Madagascar group 1 crown age	21 (10, 32)	28 (11, 45)
5	Madagsacar group 2 vs rest (*Cicadella* + New World)	72.5 (54, 91)	89 (50, 128)
6	Madagascar group 2 crown age	59 (39, 79)	71 (37, 106)
7	New World endemics +Cicadella	70.5 (52, 89)	85.5 (47, 124)
8	New World endemic crown age	61.5 (45, 78)	76 (42, 110)

Chapter 5

Taxonomic revision and phylogeny of an endemic leafhopper genus *Nesocerus* (Hemiptera: Cicadellidae: Idiocerinae) from Madagascar[1]

Abstract

The Malagasy endemic genus *Nesocerus* Freytag & Knight, 1966 is reviewed. A key is provided for the 35 recognized species, of which 29 are described as new. Diganosis and description along with illustration of both taxonomic characters and habitus are provided for all new species and holotypes of five of previously described species. *N. trimaculatus* Freytag & Cwikla is redescribed and its habitus and aedeagus are illustrated. Phylogenetic analysis of 65 adult morphological characters supports the monophyly of the genus with strong branch support. Within *Nesocerus*, the analysis recovered two sister clades, one comprising species mostly distributed in the eastern rainforests of Madagascar, and the other including species mainly restricted to the dry western region of Madagascar. The analysis did not resolve the relationship of *Nesocerus* to other Idiocerini unambiguously, but some unique derived features of the forewing and male genitalia suggest that the closest relatives of *Nesocerus* may include taxa from South Asia and South America.

Keywords: Homoptera, Auchenorrhyncha, morphology, taxonomy, distribution

Introduction

Freytag & Knight (1966) described the genus *Nesocerus* from Madagascar, with *N. spurus* as the type species. The genus was distinguished based on the presence of three apical cells bordering the appendix of the forewing, the third apical cell of the hindwing being longer than the second, and the presence of macrosetae on the dorsoventrally flattened male subgenital plates. The species *spurus*, *chelatus*, *acuminatus*, *tetanus* and *duospinus* were also described and figured by Freytag & Knight (1966). Evans (1953) had previously described two species of Malagasy idiocerine leafhoppers, *Idiocerus madagascariensis* and *I. clavopuncatatus*, but Freytag & Knight (1966) transferred these two species, known only from females, to *Nesocerus*. Freytag &

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Cwikla (1984) described *N. trimaculatus*, thereby increasing the number of species of the genus to eight.

The recent Terrestrial Arthropods of Madagascar inventory project conducted by the California Academy of Sciences and Tsimbazaza Botanical and Zoological Park has revealed that large numbers of Malagasy leafhopper species, including many species of *Nesocerus*, remain to be described. In this paper we review the taxonomy of *Nesocerus*, analyze the phylogenetic relationships among species, describe 29 new species with illustrations, and provide a key for identification of all known species of the genus based on external and male genitalia characters.

Materials and Methods

Specimens studied are primarily from material collected during the Terrestrial Arthropod Inventory of Madagascar project conducted by the California Academy of Sciences (CAS) and Tsimbazaza Botanical and Zoological Park (PBZT). Holotypes of five of the eight described species, *N. acuminatus* Freytag & Knight, *N. chelatus* Freytag & Knight, *N. clavopunctatus* (Evans), *N. madagascariensis* (Evans) and *N. spurus* Freytag & Knight, borrowed from the Muséum National d'Histoire Naturelle, Paris (MNHN), were examined and habitus photos are provided (Fig.5.1).

Morphological terminology follows Davis (1975), Linnavuori (1959) and Dietrich (2005) for habitus and genitalia characters except for leg chaetotaxy, which follows Rakitov (1998). Habitus photos were taken using a Microptics imaging system (Photografix, Richmond, Virgina). Illustrations of genitalia were made using a camera lucida attached to a compound microscope and line drawings were traced using Adobe Illustrator ver. 12.0.1.

Holotypes and paratypes are deposited in the California Academy of Sciences (CAS) and Illinois Natural History Survey (INHS) respectively, unless otherwise indicated. The label information is provided as 'materials examined'. The numbers prefixed with "MA" and "BLF" indicate collection event codes and those with the prefix "CASENT" are unique identification numbers for the individual specimens.

Phylogenetic analysis

A phylogenetic analysis was performed to test monophyly of the genus and to examine relationships among the species of *Nesocerus*.

Taxa and characters

Taxa included 41 species including 31 species of *Nesocerus* and 10 outgroup taxa. Among previously described species of the genus, two, *N. trimaculatus* Freytag and Knight and *N. tetanus* Freytag & Cwikla, were included in the analysis. Outgroup taxa comprise representatives of other idiocerine genera from both Madagascar and other continents including Africa, South America, and Asia. Sixty five characters of adult morphology were scored from direct examination of specimens. Characters and character states are listed in Appendix 1. All character states are unordered and weighted equally. The data matrix is shown in Appendix 2. Question marks in the matrix refer to missing data or inapplicable characters.

Analysis

PAUP* version 4.0 (Swofford 2001) was used for maximum parsimony analysis using a heuristic tree search with random addition sequence (1000 replicates) and TBR branch swapping. Characters that were inapplicable to some taxa were coded as ambiguous. For polymorphic taxa, all observed states were scored. Node support was estimated by calculating decay indices (Bremer 1994).

Lists of apomorphies were produced under ACCTRAN (accelerated transformation) optimization using PAUP 3.1.1 to avoid a bug in PAUP* 4.0b10, which produces inaccurate apomorphy list. The apomorphy list is given as Appendix 3.

Results

Taxonomy

Genus *Nesocerus* Freytag & Knight 1966

Type species *Nesocerus spurus*, Freytag & Knight, by original designation.

Length of male, 3.25–6.6 mm; female, 4–7 mm.

Ochraceous with black markings on crown, pronotum, mesonotum and face. Forewings with basal half fuscous, with markings along middle or costal margin. Leg yellowish or stramineous; usually with black markings along margins.

Head wider than pronotum. Length of scutum almost equal to combined length of crown and pronotum. Head, pronotum and scutum shagreened. Antennae not expanded apically or subapically. Ocelli on face equidistant from imaginary midline and inner margin of compound eye. Frontoclypeus wider than length. Laterofrontal sutures extending to corresponding ocellus, more or less straight. Clypeogenal suture not reaching antennal ledge. Lorum not extending to

apex of clypellus; usually separated from facial margin throughout length. Clypellus usually constricted at middle. Pronotum with hind margin straight. Forewings with three apical cells bordering broad appendix; with two subapical cells, first cell open, second cell open or closed; clavus with two veins. Hind femur setal formula 2+1(in *N. serratus* sp. nov. 2+0). Hind tibia flattened. Hind tarsomere I without dorsoapical setae, with two ventral longitudinal rows of short setae, pecten with lateral setae longer than platellae (Fig. 5.2 A & B).

Male pygofer with long or short posterodorsal process; posterolateral margin with setae; dorsolateral fold absent. Anal segment loosely attached to pygofer. Subgenital plate dorsoventrally flattened, longer than pygofer; in ventral aspect narrowed towards apex, with prominent macrosetae along margin, fine setae long or short, restricted to dorsum of apex and along lateral margin. Style without lateral lobe, apophysis curved dorsally. Connective semicircular with two dorsal keels, T-, V-or Y-shaped; articulated or fused to basal part of aedeagus. Aedeagus with socle developed or not; shaft cylindrical or laterally compressed, extending dorsad or anterodorsad, with or without apical or basal processes. Gonopore apical or subapical.

Female ovipostitor extending beyond pygofer. First valvulae blade shaped, curved slightly dorsally with strigate dorsal sculptured area.

Distribution: Widely distributed in Madagascar.

Discussion. The genus can be distinguished by the following combination of characters: forewing with three apical cells bordering appendix; hind tarsomere I without pair of dorsoapical setae, plantar surface of hind tarsomere I with two ventral longitudinal rows of short setae, platellae shorter than lateral setae; subgenital plate depressed with macrosetae on ventral surface.

Key to males of *Nesocerus*

1. Connective Y-, V- or T-shaped, articulated with aedeagus; style in lateral view slender with apodeme narrow and apex hooked (Fig. 5.12T, U)…**2**

—Connective semicircular (Fig. 5.6G), fused with aedeagus; style in lateral view with apodeme broad (Fig. 5.6F) and apex variable .. **12**

2. Head with broad black marking restricted to crown, pronotum and scutum, face unmarked (Fig. 5.5K,L); aedeagus with apical forked single process and basal dorsally directed pair of processes (Fig. 5.13R); shaft apex in posterior view broad and round (Fig. 5.13S)…26. ***N. latus*, sp. nov.**

—Head without marking as above, face with scattered black markings; aedeagus not as above…..**3**

3. Forewing with dark brown band along middle of costal margin (Fig. 5.5Q); aedeagus in posterior view with lateral flange extending from base nearly to apex, gradually narrowed towards apex (Fig. 5.14S)……. 29. ***N. convolutus*, sp. nov.**

— Forewing unmarked; aedeagus not as above…4

4. Aedeagus with lateral or basal processes….5

—Aedeagus without processes… ***N. duospinus* Freytag & Knight**

5. Aedeagal shaft with five processes…..**6**

— Aedeagal shaft with fewer than five processes…**7**

6. Pygofer with elongate process arising basiventrally, extending along posterior margin (Fig. 5.11O); aedeagal shaft long and slender with apical pair of processes shorter than subapical ones (Fig. 5.9R, S)… 20. ***N. quinquespinosus*, sp. nov.**

—Pygofer with short process arising dorsally, extending mesad; aedeagal shaft short and broad with apical and subapical pairs of processes almost equal in length (Fig. 5.15 C, D) ……30. ***N. trimaculatus* Freytag & Cwikla**

7. Aedeagus with dorsal apodeme reduced (Fig. 5.12R); pronotum with pair of circular median spots (Fig. 5.5E)… 23. ***N. compressus*, sp. nov.**

—Aedeagus with dorsal apodeme well developed (Fig. 5.10K); pronotum with many markings…**8**

8. Aedeagal shaft with basal or subbasal processes extending dorsad or posterodorsad (Fig. 5.12K, 5.14D)…..**9**

—Aedeagal shaft with apical processes extending ventrad or ventrolaterad (Fig. 5.14K)…. **11**

9. Aedeagal shaft with single process, arising near shaft midlength, extending close to shaft throughout length (Fig. 5.12K)…22. ***N. uniprocessus*, sp. nov.**

—Aedeagal shaft with two or three processes, arising near base of shaft (Fig. 5.14D) ……**10**

10. Aedeagal shaft with single pair of processes; style with hooked apex.. ***N. tetanus*** **Freytag & Knight**

—Aedeagal shaft with single ventral process and pair of dorsal processes (Fig. 5.14 D, E); style with broadly curved apex (Fig. 5.14F).. 27. ***N. basiprocessus,*** **sp. nov.**

11. Aedeagal shaft laterally flattened, with symmetrical apical processes; pygofer with process along posterior margin… **12**

—Aedeagal shaft tubular, with asymmetrical apical or subapical processes; pygofer without processes along posterior margin…. **13**

12. Apical processes of aedeagal shaft elongate extended more than half distance to base of shaft, with lateral margins serrate; shaft in posterior view with crenulate lateral margin (Fig. 5.14K, L)….. 28**. *N. serratus,*** **sp. nov.**

—Apical processes of aedeagal shaft short, extended no more than one third distance to base of shaft, with lateral margin smooth; shaft in posterior view with lateral margin smooth (Fig. 5.13K, L)… 25. ***N. breviprocessus*****, sp. nov.**

13. Lorum with median black spot; clypeogenal suture bordered with black stripe (Fig. 5.5H); aedeagal shaft in lateral view broad, bent anterad near middle, then tapered towards apex, with two short apical processes (Fig. 5.13D, E) … 24. ***N. flexus,*** **sp. nov.**

—Lorum and clypeogenal suture unmarked; aedeagal shaft in lateral view slender, uniformly wide throughout length, with one short apical and one elongate subapical process (Fig. 5.12D, E).. 21. ***N. asymmetricus*****, sp. nov.**

14. Forewing with black band along clavus extending to apex (Fig. 5.4E); style with digitiform processes near apex (Fig. 5.9 M, N)… 13. ***N. dentatus*****, sp. nov.**

— Forewing without coloration as above, style without digitiform process …..**15**

15. Aedeagus with extended gonopore membrane and apical processes with microtrichiae (Fig. 5.7S, T) ….8. ***N. littoralis,*** **sp. nov.**

— Aedeagus not as above….**16**

16. Head with three broad black stripes extending to face (Fig. 5.3A, 5.4K)…**17**

— Head without broad stripes as above…**19**

17. Aedeagus with apical processes… **18**

— Aedeagus with basal process (Fig. 5.10K), style apex acuminate with slight bend subapically (Fig. 5.10M)…16. ***N. tristriatus*****, sp. nov.**

18. Aedeagal shaft in lateral view broad at middle, with long straight apical processes extending beyond midlength ….. ***N. acuminatus*** **Freytag & Knight**

— Aedeagal shaft in lateral view uniformly broad throughout length, with short sinuate apical processes not extending beyond midlength (Fig. 5.6D, E) ….. 1. ***N. trilineatus,*** **sp. nov.**

19. Pygofer posterodorsal process elongate and forked in posterior view (Fig. 5.7I), aedeagus with two pairs of apical processes … **20**

— Pygofer with posterodorsal process not forked in posterior view, aedeagus with pair of apical processes …..…**22**

20. Pronotum with single median black spot (Fig. 5.4G), crown with median pair of black spots, aedeagus with ventrolaterally directed processes ….**21**

— Pronotum and crown mottled with light brown or black markings (Fig. 5.3M); aedeagus with dorsolaterally directed processes (Fig. 5.7L, M) …..7. ***N. bifurcatus,*** **sp. nov.**

21. Style with abruptly acuminate apex (Fig. 11F, G); aedeagus with pair of subapical process short, straight and apical pair long, curved (Fig. 5.11D, E)……18. ***N. nanus,*** **sp. nov.**

— Style with gradually tapered acute apex (Fig. 5.9T); aedeagus with both pairs of processes long, subapical pair crossing midline (Fig. 5.9R, S) ……14. ***N. unimaculatus,*** **sp. nov.**

22. Pygofer with single posterodorsal process (Fig. 5.8A).. **23**

— Pygofer with more than one posterodorsal process (Fig. 5.10A, Fig. 5. 8H)… **32**

23. Style apex in lateral view broad, laterally compressed and abruptly curved dorsad (Fig. 5.8F); connective with anterior margin shallowly concave (Fig. 5. 8G)……9. ***N. fasika,*** **sp. nov.**

— Style apex not as above; connective with anterior margin deeply concave…..**24**

24. Subgenital plate with single row of macrosetae; pygofer with apex of posterodorsal process slightly hooked (Fig. 5.8O) ……. **25**

— Subgenital plate with more than one row of macrosetae; Pygofer with apex of posterodorsal process truncate or acute …. **26**

25. Aedeagus in lateral view with ventrally directed long recurved apical processes (Fig. 5.8R, S); style apex in lateral view with preapical tooth (Fig. 5.8U)…. 11. ***N. recurvus,*** **sp. nov.**

— Aedeagus in lateral view with anterodorsally directed long curved apical processes (Fig. 5.9D, E); style apex in lateral view gradually tapered and sinuate, without preapical tooth (Fig. 5.9F, G) ….. 12. ***N. dorsalis,*** **sp. nov.**

26. Subgenital plate in lateral view with subdorsal process; forewing with orange spots… ***N. spurus*** **Freytag & Knight**

— Subgenital plate in lateral view without subdorsal process; forewing without spots…**27**

27. Pygofer with single posterodorsal process abruptly bent ventrad (Fig. 5.7A); subgenital plate in ventral view with mesal margin slightly convex at middle (Fig. 5.7B).. **28**

— Pygofer with posterodorsal process gradually curved ventrad; subgenital plate in ventral view with mesal margin straight … **29**

28. Subgenital plate with apex curved dorsad, style in lateral view with apex abruptly acute (Fig. 5.7B); aedeagus in lateral view with long apical processes strongly recurved (Fig. 5.7D, E)….6. ***N. orbiculatus,*** **sp. nov.**

— Subgenital plate with apex not curved dorsad, style in lateral view with apex acuminate; aedeagus in lateral view with short curved apical processes (Fig. 5.11K, L) ……. 19. ***N. mananarensis,*** **sp. nov.**

29. Clypellus profile convex; subgenital plate with long fine setae twice length of macrosetae… **30**

— Clypellus profile straight; subgenital plate with short fine setae equal or shorter than macrosetae.. **31**

30. Aedeagal shaft with angulate lateral flange near base; socle in posterior view narrowest towards base (Fig. 5.6V, W)….4. ***N. angulatus,*** **sp. nov.**

— Aedeagal shaft without angulate lateral flange near base; socle in posterior view parallel-sided (Fig. 5.6Z, A1)……5. ***N. sclerophyllus,*** **sp. nov.**

31. Aedeagus with ventrally directed long apical processes (Fig. 5.6L, M); forewing with r-m1 crossvein …… 2. ***N. ranomafanaensis,*** **sp. nov.**

— Aedeagus without long apical processes (Fig. 5.6P, Q); forewing without r-m1 crossvein …….3. ***N. affinis,*** **sp. nov.**

32. Aedeagus without apical processes; style in lateral view with apex narrow and acuminate without process … ***N. chelatus,*** **Freytag & Knight**

— Aedeagus with apical processes; style apex in lateral view with one or more preapical processes, broad and abruptly curved dorsad or narrow …. **33**

33. Style apex in lateral view broad abruptly curved dorsad beyond middle; aedeagal shaft long and slender with long apical processes; connective with anterior margin shallowly concave … **34**

— Style apex in lateral view narrow, gradually curved dorsad near middle (Fig. 5.10S); aedeagal shaft short and stout with short apical processes (Fig. 5.10R); connective with anterior margin deeply concave (Fig. 5.10T) …17. ***N. brevipinguis*, sp. nov.**

34. Length including forewing more than 4 mm; pronotum with broad black markings (Fig. 5.4I) …. 15. ***N. nigrum*, sp. nov.**

— Length including forewing less than 4 mm; pronotum with light brown markings (Fig. 5.3S) … 10. ***N. tulearensis,* sp. nov.**

1. *N. trilineatus,* sp. nov.

Fig. 5.3A, B; Fig. 5.6A–H; Fig. 5.16A, B

Diagnosis

This species is similar in habitus to *N. tristriatus* in the presence of three black longitudinal stripes on the head, but males can be differentiated from the latter by the presence of aedeagal processes.

Description

Length of male, 4.3 mm; female, 5.5–6.2 mm.

Coloration. Crown, pronotum and scutum yellow with three longitudinal black stripes extending to face, middle one narrow not extending posterad of scutellur suture. Antennae with scape and pedicel light brown, flagellum dull yellow. Frontoclypeus with median longitudinal black stripe. Clypeogenal suture bordered with black marking. Gena with black marking. Clypellus completely black. Forewing with dark stripe bordering clavus. Femur with pale brown lines along anterior and posterior margins.

Structure. Clypellus with sides concave, apex narrower than base; in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein absent. Metatibial setal rows posterodorsal (PD), anterodorsal (AD), and anteroventral (AV) with 14–15, 10 and 11–12 macrosetae, respectively.

Male genitalia. Pygofer in lateral view with falcate posterodorsal process with apex slightly truncate; posterolateral margin with long setae. Subgenital plate in lateral view depressed

basally, broadened near apex, long fine setae along lateral margin and dorsum of apex; in ventral view lateral margin strongly convex towards base, evenly narrowed toward apex, with submarginal row of macrosetae along apical half. Style in lateral view with broad apodeme; apophysis abruptly tapered and curved dorsad beyond middle, apex acuminate, in posterior view long. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view broad, straight; apex in posterior view narrow, rounded, with pair of long ventrolaterally directed sinuate processes extending almost half length of shaft; gonopore apical.

Female genitalia. Second valvulae in lateral aspect blade shaped, preapical portion slightly widened, then narrowed to apex; apical third of dorsal margin with teeth widely spaced basally and narrowly spaced distally, extending to ventral apical margin; ducts conspicuous.

Materials examined. Holotype male, MADAGASCAR: Province d'Antananarivo, 3 km 41° NE Andranomay, 11.5 km, 1300 m, 18°28'24" S, 47°57'36" E, 5-13. XII. 2000, Fisher, Griswold et al., BLF2375, CASENT 5501158 (CAS). *Paratypes*: 2 females, same data as holotype (CAS, INHS); 2 females, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, elev 1130 m, 22°15'3" S, 47°24'25" E, 21-24. XII. 2001, Harin'Hala, R., malaise, mixed tropical forest, MA-02-09B-08 (CAS, INHS).

Etymology. The specific epithet refers to the three longitudinal markings on the head.

2. *Nesocerus ranomafanaensis*, sp. nov.

Fig.5.2 C, D; Fig.5.6I–O

Diagnosis

This species is closely similar in habitus and male genitalia to *N. affinis*, but can be distinguished by the presence of a pair of long lateral processes on the aedeagal shaft and the presence of crossvein r-m_1 on the forewing.

Description

Length of male, 4.4–4.6 mm; female, 5 mm.

Coloration. Crown with three to four black markings extending to face reaching antennal ledge, median spots usually closer, forming v-shaped marking extending laterad to ocellus. Antennae with scape and pedicel light brown, flagellum dull yellow. Frontoclypeus with median broad black T- shaped stripe; clypeogenal and laterofrontal sutures bordered with black marking. Gena

unmarked. Clypellus completely black or with central small yellow spot. Pronotum with black median markings arranged transversely along anterior region, a few irregular black markings close to posterior margin. Mesonotum with basal black triangle bordered by light brown line, not extending beyond scutellar suture. Forewing mostly hyaline. Legs yellow, fore- and hind femur with pale brown lines along anterior and posterior margins.

Structure. Clypellus with sides concave, apex as wide as base; in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein present. Metatibial setal rows PD, AD, and AV with 13–14, 9 and 11 macrosetae, respectively.

Male genitalia. Pygofer, style and connective as in *N. trilineatus*. Subgenital plate in lateral view depressed basally, broadened near apex and short fine setae almost equal to length of macrosetae restricted to dorsum of apex; in ventral view lateral margin strongly convex towards base, evenly narrowed toward apex, with biseriate submarginal macrosetae extending along distal two thirds. Aedeagus in lateral view with atrium broad; socle well developed; shaft in lateral view directed dorsally, broad, nearly straight and uniform in width, apex in posterior view acuminate, with pair of long ventrolaterally directed subapical processes extending to about midlength of shaft, apex of processes gradually curved laterad; gonopore subapical on posterior surface of shaft basad of processes.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59” S, 47°25’26” E, 12-19. II. 2002, R. Harin’Hala, malaise, secondary forest, MA-02-09C-16 (CAS). *Paratypes*: 4 males, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59” S, 47°25’26” E, 14-23. IV. 2002, R. Harin’Hala, malaise, secondary forest, MA-02-09C-25 (CAS, INHS); 2 males, 1 female, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Vohiparara at broken bridge, 1110 m, 21°13’34” S, 47°22’11” E, 28. XI – 6. XII. 2001, R. Harin’Hala, malaise trap in high altitude rainforest, MA-02-09A-05 (CAS, INHS); 1 male, MADAGASCAR: Fianarantsoa, Parc National Ranomafana, 2.3 km N village Vohiparara, 1100 m, 21°13’26” S, 47°22’51” E, 18. IV. 1998, C.E. Griswold, D. H. Kavanaugh, N.D. Penny, M. J. Raherilalao, J. S. Ranorianarisoa, Jere Schweikért. & D. Ubick, CASENT 303332 (CAS); 1 male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 21 – 28. I. 2002, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-13 (INHS).

Etymology. The specific epithet is derived from the type locality "Ranomafana."

3. *Nesocerus affinis,* sp. nov.

Fig.5. 3I, J; Fig.5. 6P, Q

Diagnosis. This species is closely similar to *N. ranomafanaensis*, but can be distinguished by the reduced or absent apical processes of the aedeagal shaft and the absence of forewing crossvein r-m_1.

Description.

Length of male, 4.6–4.8 mm; female, 5 mm

External characters and coloration as in *N. ranomafanaensis*.

Male genitalia closely resembling those of *N. ranomafanaensis*, but aedeagal shaft with processes absent or reduced to tiny lobes.

Materials examined. Holotype male, MADAGASCAR: Province d'Antananarivo, 3 km 41° NE Andranomay, 11.5 km, 1300 m, 18°28'24" S, 47°57'36" E, 5-13. XII. 2000, Fisher, Griswold et al., BLF2375, CASENT5502778 (CAS). *Paratypes*: 2 males (CASENT5501890, CASENT5502683), 1 female (CASENT5501002), same data as holotype (CAS, INHS); 1 male, MADAGASCAR: Province d'Antsiranana, Parc National Montagne d'Ambre, 12.2 km 211°SSW Joffreville, 1300 m, 21°35'47"S, 49°9'34" E, 2-7. II. 2001, Fisher, Griswold et al., BLF2853 (INHS).

Etymology. The specific epithet is derived from the Latin *affinis* meaning similar, referring to similarity with *N. ranomafanaensis*.

4. *N. angulatus,* sp. nov.

Fig.5. 3E, F; Fig.5. 6R–Y

Diagnosis. This species can be distinguished from others by the following combination of characters: convex lateral profile, attenuate posterodorsal process of pygofer, aedeagus with angulate lateral flange near the base and shape of socle in posterior view narrower towards base. This species is similar to *N. sclerophyllus* in habitus and male genitalia, but can be distinguished by the shape of the socle and presence of a lateral flange on the aedeagus.

Description

Length of male, 4.1 mm.

Coloration. Crown with three irregular black markings extending to face reaching ocelli. Antennae with scape and pedicel light brown near base, flagellum dull yellow. Frontoclypeus with median broad black longitudinal stripe joining apically arc-shaped stripe below ocelli, with additional pair of lateral longitudinal stripes; clypeogenal sutures bordered by black stripe. Gena with black marking below antennal ledge. Lorum with black marking along side bordering clypellus. Clypellus black with median small yellow spot near the base. Pronotum with irregular black markings with median longitudinal narrow stripe. Mesonotum with black basal triangle, median diverging black lines not extending posterad of scutellar suture. Forewing mostly hyaline. Legs yellow with pale brown lines along anterior and posterior margins. Mid femur with black stripe along ventral surface near apex.

Structure. Clypellus with sides almost straight, apex narrower than base, in profile convex. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein absent. Metatibial setal rows PD, AD, and AV with 17-20, 8 and 11 macrosetae, respectively.

Male genitalia. Pygofer broad with posterodorsal process tapered and curved slightly ventrad, apex of process slightly truncate; posterolateral margin with long setae. Subgenital plate, style and connective as in *N. trilineatus*. Aedeagus in lateral view with atrium narrow; socle well developed; shape of preatrium in posterior view tapered towards base, shaft in lateral view broad, curved slightly anterad, slightly tapered towards apex; shaft in posterior view acuminate, with pair of short ventrolaterally directed apical processes not extending half of the shaft, with pair of angulate lateral flange near base. Gonopore subapical on posterior surface of shaft basad of processes.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59” S, 47°25’26” E, 22-28. XI. 2001, R. Harin’Hala, malaise, secondary tropical forest, MA-02-09C-04 (CAS). *Paratype*: 1 male, same data as holotype except 31. III- 7. IV. 2002, MA-02-09C-23 (INHS).

Etymology. The specific epithet is derived from the Latin word *angulus*, referring to the angulate lateral flange on the aedeagus.

5. *N. sclerophyllus,* sp. nov.

Fig.5. 3K, L; Fig.5. 6Z, A1; Fig.5. 16C, D

Diagnosis. Males of this species are similar to those of *N. angulatus* in habitus and male genitalia morphology, but may be distinguished by the uniform width of the preatrium in posterior view and the presence of long apical aedeagal processes reaching almost the midlength of the shaft.

Description.

Length of male, 4 mm; female, 4.9 mm

Coloration, structure similar to that of *N. angulatus*.

Male genitalia. Pygofer, subgenital plate, style, connective as in *N. angulatus*. Aedeagus in lateral view with atrium narrow, socle well developed, preatrium in posterior view with apex as broad as base, shaft in lateral view broad, curved slightly anterad, slightly tapered towards apex; apex in posterior view acuminate, with pair of long ventrally directed apical processes reaching almost middle of the shaft, then curved laterad. Gonopore subapical.

Female genitalia. Same as in *N. trilineatus*

Materials Examined. Holotype male, MADAGASCAR: Province Antananarivo, 46 km NE of Ankazobe, Ambohitantel, 700 m, 18°11’52” S, 47°16’53” E, 15. X. – 1. XI. 2004, M. Irwin, F. Parker & R. Harin'Hala, malaise trap, in sclerophyll forest, MA-27-19 (CAS). *Paratypes*: 1male, 1 female, MADAGASCAR: Province Antananarivo, 46 km NE of Ankazobe, Ambohitantel, 700 m, 18°11’52” S, 47°16’53” E, 29. XI. – 6. XII. 2003, M. Irwin, R. Harin'Hala, malaise trap, in sclerophyll forest, MA-27-08 (INHS, CAS).

Etymology. The specific epithet refers to the sclerophyll habitat of the type locality.

6. *Nesocerus orbiculatus,* sp. nov.

Fig.5. 3G, H; Fig.5. 7A–G

Diagnosis. This species is similar to *N. mananarensis* in habitus and in the abruptly bent pygofer posterodorsal process and slightly convex mesal margin of the subgenital plate. It can be separated by the following combination of characters: frontoclypeus with arc shaped marking; male subgenital plate with apex abruptly curved dorsad; style with dorsal and ventral spine near apex and apical processes of aedeagus long and recurved.

Description

Length of male, 4.5–4.8 mm; female, 5.3–5.4 mm.

Coloration. Crown with three black or brown markings; middle marking usually v-shaped, joining tranverse irregular patch near anterior margin of face that extends to laterofrontal suture. Antennae with scape yellow, pedicel and flagellum light brown. Frontoclypeus with median longitudinal irregular black stripe surrounded by irregular black arc shaped marking; clypeogenal sutures bordered by black stripe. Gena with black marking below antennal ledge. Clypellus bordered by broad black stripe except along apex, with yellow region along middle. Pronotum with numerous irregular black markings with median longitudinal narrow stripe. Mesonotum with pair of basal black triangle, usually with yellow region at middle and a pair of median black marking near scutellar suture. Scutellum with black macula. Forewing with claval vein white with alternate brown coloration. Fore- and mid femur with lateral black stripes near base and apex, hind femur rarely with lateral black markings.

Structure. Clypellus with sides concave, apex as wide as base, in profile slightly convex. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein present. Metatibial setal rows PD, AD, and AV with 15, 8–10 and 9–11 macrosetae respectively.

Male genitalia. Pygofer broad with posterodorsal process tapered, directed ventrad; posterolateral margin with short setae. Subgenital plates in lateral view depressed basally, apex abruptly bent dorsad, with short fine setae restricted to dorsum of apex; in ventral view widest near midlength, lateral margin broadly convex tapering to broadly round apex, macrosetae confined to distal two thirds along lateral and mesal margins. Style in lateral view with broad apodeme; apophysis sinuate in dorsal margin, nearly uniform in width, curved dorsad, apex slightly narrowed with large dorsal and short ventral spine. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view slender, uniform in width along its length, curved slightly anterad; apex in posterior view bifurcated with long terminal processes, curved venterolaterad, then extending anterodorsad, in nearly complete circle in lateral view; gonopore apical on posterior surface of shaft.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59” S, 47°25’26” E, 22-28. XI. 2001, R. Harin’Hala, malaise, secondary forest, MA-02-09C-04 (CAS). *Paratypes*: 1 female,

MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59” S, 47°25’26” E, 14-23. IV. 2002, R. Harin’Hala, malaise, secondary forest, MA-02-09C-25 (CAS); 2 males, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Vohiparara at broken bridge, 1110 m, 21°13’34” S, 47°22’11” E, 4-12. II. 2002, R. Harin’Hala, malaise trap in high altitude rainforest, MA-02-09A-15 (CAS, INHS); 1 male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59” S, 47°25’26” E, 24. VII - 4. VIII. 2002, R. Harin’Hala, malaise, secondary forest, MA-02-09C-36 (CAS); 2 females, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 21- 24.II. 2001, R. Harin’Hala, malaise, mixed tropical forest, MA-02-09B-08 (CAS, INHS); 1 female, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 12-19.II. 2002, R. Harin’Hala, malaise, mixed tropical forest, MA-02-09B-16 (CAS); 2 females, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Vohiparara at broken bridge, 1110 m, 21°13’34” S, 47°22’11” E, 28. XI- 6. XII. 2001, R. Harin’Hala, malaise trap in high altitude rainforest, MA-02-09A-05 (CAS, INHS); 1 female, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 28. I. - 4. II. 2002, R. Harin’Hala, malaise, mixed tropical forest, MA-02-09B-14 (INHS).

Etymology. The specific epithet is derived from the Latin word *orbiculatus* meaning circular, in reference to the curved lateral processes of the aedeagus.

7. *Nesocerus bifurcatus* sp. nov.

Fig.5. 3M, N; Fig.5. 7H–O

Diagnosis. This species has the following unique combination of characters: pygofer process forked in posterior view; subgenital plate with biseriate row of macrosetae along lateral submargin; style robust, almost straight throughout its length with short acute apical process and aedeagal shaft slender, with two pairs of symmetrical apical processes extending laterad.

Description

Length of male, 4.7 mm.

Coloration. Crown, pronotum with irregular black markings. Mesonotum with basal light brown triangle bordered by dark marking; median black lines extending to scutellum. Antennae with

scape dull yellow; pedicel and flagellum light brown. Frontoclypeus with median stripe; with yellow demarcation along facial sutures. Gena with black marking. Clypellus with median yellow region bordered by black stripe. Forewing with claval vein white with alternate brown coloration. Legs with pale brown lines along dorsal and ventral margins.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein present. Metatibial setal rows PD, AD, and AV with 14–15, 10 and 11–12 macrosetae respectively.

Male genitalia. Pygofer broad with falcate posterodorsal process, apex of process bifid; posterolateral margin with short setae. Subgenital plate in lateral view depressed basally, broadened near apex, with short fine setae confined to lateral margin and dorsum of apex; in ventral view lateral margin strongly convex towards base, evenly narrowed toward apex, with submarginal row of macrosetae along apical half. Style in lateral view with broad apodeme; apophysis slightly narrowed and curved dorsad, uniformly broad throughout its length with apical tooth. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle poorly developed; shaft in lateral view slender, broadly curved near the base, narrowed towards apex; apex in posterior view narrow, bifurcated apically and subapically; gonopore situated posteriorly below the subapical process.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 26 – 31. III. 2002, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-22 (CAS). Paratype one male (genitalia damaged), MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 21 – 28. I. 2002, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-13 (INHS).

Etymology. The specific epithet refers to the twice-forked aedeagus.

8. *N. littoralis*, sp. nov.

Fig.5. 3O, P; Fig.5. 7P–V

Diagnosis. This species has the following unique combination of characters: pygofer without elongate process; style slender with pointed sinuate apex; aedeagus with gonotheca membrane projected and clothed with microtrichia and shaft with laterally directed apical processes bearing microtrichia.

Description.

Length of male, 4.4–4.6 mm.

Coloration. Crown with three light brown to black markings, central one usually V-shaped extending to face reaching antennal ledge. Antennae with scape and flagellum yellow. pedicel light brown or black. Frontoclypeus with median black longitudinal stripe, lateral longitudinal stripe along muscle impression. Gena usually without any marking, in some specimens with dull yellow marking below eye margin. Clypellus usually black with central elongate yellow spot. Pronotum with median short black stripe, usually lateral six spots, 2 near lateral margin, median 4 spots, in some connected forming stripe Mesonotum with bright orange basal triangle with black border, median black lines extending beyond scutellar suture. Forewing with claval vein white with alternate brown coloration. Legs yellow with pale brown lines along dorsal and ventral margins. Fore- and hind femur with black stripe along dorsal margin.

Structure. Clypellus with sides concave, apex wider than base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein present. Metatibial setal rows PD, AD, and AV with 13–14, 9 and 11 macrosetae respectively.

Male genitalia. Pygofer broad, with short posterodorsal projection; posterolateral margin with short setae. Subgenital plate in lateral view depressed basally, broadened near apex; in ventral view lateral margin moderately convex towards base, evenly narrowed toward apex, with row of macrosetae scattered along apical half. Style in lateral view with narrow apodeme; apophysis uniformly wide throughout its length, curved dorsad beyond middle, apex pointed. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium narrow and elongate; socle poorly developed; shaft in lateral view broad, nearly straight, gradually tapered towards apex; apex in posterior view with pair of laterally directed terminal processes bearing microtrichia scattered along dorsal surface; gonopore situated apically on posterior surface, with gonopore membrane projected, extending to base of shaft, with microtrichia.

Material examined. Holotype male, MADAGASCAR: Province d'Antsiranana, Sakalava Beach, dwarf littoral forest, 10 m, 12°15'46" S, 49°23'51" E, 16-31. V. 2001, M. Irwin, R. Harin'Hala, malaise trap, across sandy trail, MA-01-04B-10 (CAS). *Paratypes*: 1 male, MADAGASCAR: Province Mahajanga, Parc National Tsingy de Bemaraha, 3.4 km 93° E. Bekopaka, Tombeau Vazimba, 50 m, 19°8'31" S, 44°49'41" E, 6-10. XI. 2001, Fisher, Griswold

et al., malaise trap, in tropical dry forest, BLF4233 (INHS); 2 males, MADAGASCAR: Province Mahajanga, Parc National de Namoroka, 16.9 km 317° NW Vilanandro, 100 m, 16°24’24” S, 45°18’36” E, 12 – 16. XI. 2002, Fisher, Griswold et al., yellow pan trap, in tropical dry forest, BLF6586 (CAS, INHS).

Etymology. The specific epithet refers to the littoral habitat of the type locality.

9. *N. fasika*, sp. nov.

Fig.5. 3Q, R; Fig.5. 8A–G

Diagnosis. This species may be distinguished by the following unique combination of characters: frontoclypeus with pair of tiny circular ivory spots near ocelli and remainder covered with dark brown mottled markings, pygofer process forked in posterior view; style abruptly curved anterodorsad beyond middle with broad laterally compressed apex; connective with dorsal margin shallowly concave and aedeagal shaft slender, sinuate near apex with laterally directed short apical process.

Description

Length of male, 4.3–4.4 mm.

Coloration. Crown mottled with light brown markings. Antennae with scape and flagellum yellow, pedicel light brown towards base. Frontoclypeus mottled with black or brown markings varying in darkness from crown to base of clypellus, two oval or circular yellow spot below ocelli. Gena with black marking. Clypellus completely black or with central small yellow spot. Pronotum with irregular black marking, lighter in posterior half. Mesonotum with basal black triangle without any border, median irregular pattern not extending beyond scutellar suture. Scutellum with light brown longitudinal lines. Forewing with claval vein white with alternate brown coloration. Legs yellow, fore- and hind femur with black stripe near base and apex, hind femur with black stripe along dorsal margin.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein present. Metatibial setal rows PD, AD, and AV with 13–14, 9 and 11 macrosetae respectively.

Male genitalia. Pygofer broad with acute posterodorsal process; posterolateral margin with short setae. Subgenital plate in lateral view depressed basally, broadened near apex, with short fine setae restricted to dorsum of apex; in ventral view with lateral margin strongly convex

towards base, evenly narrowed toward apex, with submarginal row of macrosetae along apical half. Style in lateral view with broad apodeme; apophysis gradually narrowed along middle, then abruptly curved anterodorsad with broad laterally compressed apex. Connective in dorsal view semicircular, broadly flattened, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view slender, S-shaped, tapered towards apex; apex in posterior view narrow, rounded, with pair of laterally directed short processes; gonopore apical.

Materials examined. Holotype male, MADAGASCAR: Province Tulear, Ifaty, near Hotel Paradisia, in coastal dunes, 9 m, 23°10'46" S, 43°37'00" E, 13. X. - 12. XI. 2001, M. E. Irwin, F. D. Parker, R. Harin'Hala, malaise trap, vegetation in sandy area, MA-02-16-01 (CAS). Paratype: one male, MADAGASCAR: Tulear Province, Beza Mahafaly Reserve, Parcelle I near research station, 165 m, 23°41'19" S, 44°35'27" E, 18 – 25. I. 2002, R. Harin'Hala, malaise trap in dry deciduous forest, MA-02-14A-12 (INHS).

Etymology. The specific epithet is derived from the Malagasy word *fasika* meaning sandy area, referring to the sandy type locality.

10. *N. tulearensis*, sp. nov.

Fig.5. 3S, T; Fig.5. 8H–N; Fig.5. 16E, F

Diagnosis. This species may be distinguished by the following combination of characters: pygofer with both dorsal and ventral process; subgenital plate with truncate apex and multiseriate submarginal macrosetae; style abruptly curved anterodorsad with broadly flattened, medially divided apex with single subapical tooth; connective with dorsal margin shallowly concave and aedeagal shaft slender with long ventrally directed terminal processes. This species is similar to *N. nigrum* in the subgenital plate, shape of connective, style apex and aedeagus, but can be distinguished by the presence of an elongate dorsal pygofer process and by the presence of light brown markings on the vertex and frontoclypeus.

Description

Length of male, 3.7–3.8 mm.

Coloration. Crown with irregular dull yellow markings. Antennae without any markings. Frontoclypeus with median irregular stripe sometimes with mottled dull yellow markings. Gena with dull yellow marking. Clypellus completely black or with central small yellow spot.

Pronotum with irregular black marking, lighter in posterior half. Mesonotum usually with light orange with black border, median light brown diverging lines. Scutellum with a pair of light brown longitudinal lines. Forewing with claval vein white with alternate brown coloration. Legs yellow, without any makings along dorsal margin of femora.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein present or absent. Metatibial setal rows PD, AD, and AV with 14–15, 9 and 9 macrosetae respectively.

Male genitalia. Pygofer broad with branched posterodorsal process; posterolateral margin with short setae. Subgenital plate in lateral view not depressed basally, uniformly wide throughout its length, with short fine setae restricted to dorsum of apex; in ventral view lateral margin broadly convex towards base, narrowed preapically with broadly rounded apex, with submarginal row of macrosetae along apical half. Style in lateral view with broad apodeme; apophysis gradually narrowed along middle, then abruptly curved anterodorsad, apex broadly flattened, medially divided, with subapical tooth. Connective in dorsal view semicircular, flattened, with median inward curvature along anterior margin, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view slender, uniformly wide throughout its length, strongly curved anterodorsad; apex in posterior view with pair of long ventrad processes extending almost half of the length of shaft; gonopore subapical, below apical processes on posterior surface of shaft.

Female genitalia. Second valvulae in lateral aspect thin, gradually narrowed to apex; apical third of dorsal margin with widely spaced, irregulary arranged teeth; ducts conspicuous.

Materials examined. Holotype male, MADAGASCAR: Tulear Province, Beza Mahafaly Reserve, Parcelle I near research station, 165 m, 23°41’19” S, 44°35’27” E, 18 - 25. III. 2002, R. Harin’Hala, malaise trap in dry deciduous forest, MA-02-14A-12 (CAS). *Paratypes*: 1 female, same data as holotype (CAS); 1 male, 2 females, same data as holotype except 22 – 29. III. 2002, MA-02-14A-21 (CAS, INHS); 3 males, same data as holotype, MA-02-14A-05 (CAS, INHS).

Etymology. The specific epithet refers to the type locality, Tulear Province.

11. *N. recurvus*, sp. nov.

Fig.5. 4A, B; Fig.5. 8O–U

Diagnosis. This species may be distinguished by the following combination of characters: falcate posterodorsal process of pygofer hooked near apex; subgenital plate with median row of macrosetae not extending beyond apical half, mesal row of macrosetae absent; style with subdorsal process and aedeagus with long recurved terminal processes.

Length of male, 4.3 mm.

Coloration. Crown with thin black markings extending to face. Antennae with scape and flagellum yellow, pedicel light brown. Frontoclypeus with irregular black pattern, median black stripe indistinct; border including region along clypeogenal suture black; gena with black marking below eye. Clypellus completely black with a central yellow spot. Pronotum with irregular black markings. Mesonotum with basal black triangle, median longitudinal thin black lines. Scutellum with two median black lines. Legs yellow with light black lines along dorsal margin. Fore- and hind femur with light brown bands near base and apex, respectively. Forewing mostly hyaline except basal half with alternate dark brown claval vein.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein absent. Metatibial setal rows PD, AD, and AV with 11, 8 and 10 macrosetae respectively.

Male genitalia. Pygofer broad with falcate posterodorsal process with acute apex; posterolateral margin with short setae. Subgenital plates in lateral view slightly depressed basally, broadened near apex; in ventral view strongly convex near base, evenly narrowed towards apex, with long fine setae along lateral margins, a few submarginal macrosetae scattered along apical half. Style in lateral view with broad apodeme; apophysis nearly uniform in width, curved dorsad beyond middle, apex acuminate dorsally with subdorsal flattened process. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium broad directed posterodorsad; socle well developed; shaft in lateral view short, broad, uniform in width along its length, curved posterad; apex in posterior view bifurcated with long terminal processes curved ventrad then recurved anterodorsad; gonopore apical on posterior surface of shaft below the processes.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 21 – 28. I. 2002, M.

Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-13 (CAS). *Paratype*: one male, same data as holotype except 26-31. III. 2002, MA-02-09B-22 (INHS).

Etymology. The specific epithet refers to the unique recurved processes of the aedeagus.

12. *N. dorsalis*, sp. nov.

Fig.5. 4C, D; Fig.5. 9A–G

Diagnosis. This species may be distinguished by the following combination of characters: falcate posterodorsal process of pygofer with hooked apex; subgenital plate with submarginal macrosetae not extending beyond apical half, without mesal row of macrosetae; style with pointed and sinuate apex and aedeagal shaft in lateral view with apical processes curved anterodorsally.

Length of male, 3.8 mm.

Coloration. Crown with three black markings extending to face. Antennae pale yellow. Frontoclypeus with discontinuous black stripe, dull yellow stripe along muscle impression. Gena will tiny black spot. Clypeogenal suture without any black marking. Laterofrontal suture bordered by black marking. Clypellus mostly dull yellow, with pair of black lines nea the base. Legs yellow without any markings. Pronotum dull yellow and grayish yellow along anterior and posterior half, respectively and with irregular black markings. Mesonotum with dull yellow basal triangle and pair of black longitudinal stripes. Legs, forewings unmarked.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein absent. Metatibial setal rows PD, AD, and AV with 16, 10 and 12 macrosetae, respectively.

Male genitalia. Pygofer with falcate posterodorsal process with acute apex, process in posterior view with rounded apex; posterolateral margin with long setae. Subgenital plates in lateral view depressed basally, broadened near apex, with long fine setae confined to lateral margin and dorsum of apex; in ventral view strongly convex near base, evenly narrowed towards apex, few submarginal macrosetae scattered along lateral margin confined to apical half. Style in lateral view with broad apodeme; apophysis nearly uniform in width, strongly curved dorsad beyond middle, apex acute. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium broad; socle well developed; shaft in lateral view long,

slender, uniform in width along its length, curved dorsad; shaft in posterior view bifurcated with long apical processes extending dorsad; gonopore apical on posterior surface of shaft.

Materials examined. Holotype male, MADAGASCAR: Province d'Antsiranana, Parc National Montagne d'Ambre, 1125 m, 12°31'13" S, 49°10'45" E, 21-26. IV. 2001, M. Irwin, R. Harin'Hala, malaise trap, MA-01-01D-08 (CAS).

Etymology. The specific epithet refers to the unique dorsally directed processes of the aedeagus.

13. *N. dentatus,* sp. nov.

Fig.5. 4E, F; Fig.5. 9H–N; Fig.5. 16G, H

Diagnosis. This species may be identified by its unique black median stripe along the clavus of the forewing extending to the apex, and by the following combination of male genitalia characters: style with numerous dentate processes near subapex and aedeagus with laterally directed short terminal processes.

Length of male, 3.9 mm; female, 4.8 mm.

Coloration. Crown with three black long markings extending to face joining transverwse black band below ocelli. Antennae with scape and flagellum light brown, pedicel black. Frontoclypeus with distinct median black stripe without any lateral black markings. Clypeogenal suture bordered by black marking,. Clypellus with yellow median region bordered by black near apex and base. Pronotum with median longitudinal stripe, two lateral irregular stripe joined anteriorly. Mesonotum with basal black triangle, median black lines extending beyond scutellar suture. Forewing mostly hyaline except broad black stripe along commisural margin extending to apex.

Structure. Clypellus with sides straight, apex as wide as base, in profile convex. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein absent. Metatibial setal rows PD, AD, and AV with 14, 8 and 9 macrosetae respectively.

Male genitalia. Pygofer broad with falcate posterodorsal process; posterolateral margin with short setae. Subgenital plates in lateral view slightly depressed basally, broadened near apex, with long fine setae along lateral margin and dorsum of apex; in ventral view strongly convex near base, evenly narrowed towards apex, with marginal and inner mesal row of macrosetae along apical half. Style in lateral view with broad apodeme; apophysis curved dorsad beyond middle, gradually tapered to acuminate apex; numerous teeth- like processes scattered near

subapical region. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium broad directed posterodorsad; socle well developed; shaft in lateral view short, broad, uniform in width along its length, curved posterad; apex in posterior view bifurcated with short terminal processes directed laterad; gonopore subapical on posterior surface of shaft.

Female genitalia. Second valvulae in lateral aspect blade shaped, preapical portion slightly widened in dorsal margin, then narrowed to apex; apical third of dorsal margin basal teeth widely spaced and distal teeth more narrowly spaced, extending to ventral apical margin; ducts inconspicuous.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Miandritsara forest, 40 km S Ambositra, 825 m, 20°47’33” S, 47°10’32” E, 26. XII. 2004 – 5. I. 2005, M. Irwin, R. Harin'Hala, malaise trap, in low altitude rainforest, MA-29-05 (CAS). *Paratypes*: 1 male, 1 female, same data as holotype (CAS, INHS).

Etymology. The specific epithet refers to the teethlike processes on the male style.

14. *N. unimaculatus,* sp. nov.

Fig.5. 4G, H; Fig.5. 9O–U

Diagnosis. This species is similar to *N. nanus* in having a pair of median black markings on the crown, a single median black spot on the pronotum, and lateral longitudinal black stripes on the scutum, but can be separated using following combination of characters: pygofer posterodorsal process forked in posterior view; style short with gradually tapered acute apex; and aedeagus with two pairs of ventrolaterally directed processes; one pair arising apically on posterior surface, other pair arising subapically on anterior surface of shaft and crossing midline.

Description.

Length of male, 4.3 mm.

Coloration. Crown with pair of median black markings extending to face. Antennae with sacpe and flagellum pale yellow, pedicel light brown. Frontoclypeus with median black stripe. Clypeogenal and laterofrontal suture bordered by black markings. Gena with black marking near eye. Clypellus completely black. Lorum with black border near apex. Pronotum with central large almost circular black spot. Mesonotum with basal black triangle extending to scutellum.

Forewing with claval vein white. Legs mostly pale yellow, hind femur with light brown patch along dorsal surface.

Structure. Clypellus with sides concave, apex wider than base, in profile flat. Rostrum extending beyond mid coxae. Forewing with with r-m_1 crossvein present. Metatibial setal rows PD, AD, and AV with 14, 9 and 8 macrosetae respectively.

Male genitalia. Pygofer broad with short falcate posterodorsal process; posterolateral margin with long setae. Subgenital plate in lateral view broadened near base, then abruptly narrowed near rounded apex, with long fine setae confined to lateral margin and dorsum of apex; in ventral view lateral margin strongly convex near base, evenly narrowed toward apex, with both outer and mesal row of macrosetae. Style in lateral view with broad apodeme; apophysis gradually tapered beyond middle, apex acuminate and curved dorsad. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view broad, uniform in width throughout its length, slightly curved anterad, narrowed near apex; apex in posterior view narrow, rounded, with pair of long ventrolaterally directed sinuate processes extending almost to midlength of shaft and pair of long ventrally directed processes arising subapically on anterior surface of shaft, crossing midline, then curved laterad; gonopore subapical on posterior surface of shaft.

Material examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 16 – 23. IV. 2002, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-25 (CAS).

Etymology. The specific epithet refers to the presence of a single spot on the pronotum.

15. *Nesocerus nigrum,* sp. nov.

Fig.5. 4I, J; Fig.5. 10A–G

Diagnosis. This species is similar to *N. tulearensis* in the shape of the subgenital plate, style, connective and aedeagus, but may be distinguished by the presence of black markings extending along the pronotum and by the absence of an elongate posterodorsal pygofer process.

Description. Length of male, 7 mm.

Coloration. Crown with three longitudinal black markings, median one v-shaped extending to face. Antennae with pedicel and flagellum brown, scape dull yellow. Frontoclypeus without median stripe, with numerous irregular black markings above antennal ledge, pair of longitudinal

lateral stripe along muscle impressions. Clypeogenal and laterofrontal suture, gena with black markings. Lorum with black line confined to base. Clypellus completely black. Pronotum mostly black with scattered yellow spots. Mesonotum with basal black triangle joined with median line not extending to scutellar suture. Scutellum with pair of black markings. Forewing hyaline with black and white claval vein. Legs pale yellow, fore-, mid femur without any marking, hind femur with thin black lines along dorsal surface.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m_1 crossvein absent. Metatibial setal rows PD, AD, and AV with 15, 8 and 9 macrosetae respectively.

Male genitalia. Pygofer with subdorsal process arising near posterior margin; posterolateral margin with long setae. Subgenital plates in lateral view weakly depressed, with short fine setae along lateral margin and dorsum of apex; in ventral view lateral margin near base strongly convex abruptly tapering towards truncate apex, two rows of macrosetae along distal two thirds of lateral margin and a few widely spaced macrosetae along mesal margin. Style in lateral view with broad apodeme; apophysis uniformly broad, curved beyond midlength to dorsally directed broad apex, apex with single basal and subbasal processes. Connective in dorsal view semicircular, fused with preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view slender, curved anterodorsad, tapered towards apex with ventrally directed long apical process reaching beyond half length of shaft; shaft in posterior view slightly narrowed near apex; gonopore subapical on posterior surface of shaft basad of processes.

Materials examined. Holotype male, MADAGASCAR: Province d'Antsiranana, Sakalava Beach, dwarf littoral forest, 10 m, 12°15'46" S, 49°23'51" E, 27. IV – 13. V. 2001, M. Irwin, R. Harin'Hala, malaise trap, across sandy trail, MA-01-04B-08 (CAS).

Etymology. The specific epithet refers to the black coloration on the pronotum.

16. *N. tristriatus*, sp. nov.

Fig.5. 4K, L; Fig.5. 10H–N

Diagnosis. This species is similar in habitus to *N. trilineatus*, but may be separated by the following combination of characters: style apex acuminate with slight bend subapically;

aedeagus without apical processes, shaft broad near apex, in caudal view with bifurcated ventrolateral flange extending from base to midlength.

Description.

Length of male, 5.6 mm.

Coloration. Crown, pronotum, scutum yellow with three longitudinal black stripes extending to face and beyond scutellar suture. Antennae with scape and pedicel black, flagellum light brown. Clypeogenal suture, laterofrontal suture, gena and lorum with black marking. Clypellus mostly black with tiny yellow spot near base. Legs yellow with black line along posterior margin. Forewing mostly hyaline.

Structure. Clypellus with sides parallel, apex narrower than base, in profile slightly convex. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 16, 9 and 12 macrosetae, respectively.

Male genitalia. Pygofer broad with falcate posterodorsal process with pointed apex in lateral view, posterolateral margin with long setae. Subgenital plate and connective similar to those of *N. trilineatus*. Style in lateral view with broad apodeme; apophysis abruptly tapered beyond middle, apex acuminate with slight bent subapically, in posterior view extending dorsad reaching beyond half of aedeagal shaft. Aedeagus in lateral view with atrium narrow, socle well developed, shaft in lateral view broad, slightly sinuate near middle, broadened near roundly flattened apex, without processes, with flange arising near base and extending to midlength of shaft, apex of flange in posterior view bifurcate, shaft in posterior view slender. Gonopore apical.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Miandritsara Forest, 40 km S Ambositra, 825 m, 20°47’33” S, 47°10’32” E, 28. I. – 9. II. 2005, M. Irwin, R. Harin'Hala, malaise trap, in low altitude rainforest, MA-29-08 (CAS).

Etymology. The specific epithet refers to the three longitudinal markings on the head.

17. *N. brevipinguis*, sp. nov.

Fig.5. 4M, N; Fig.5. 10O–T

Diagnosis. This species may be distinguished by the following combination of characters: pygofer with subdorsal process truncate; style abruptly bent dorsad near middle with dorsal acute process and aedeagus short, stout, sinuate with truncate apex and ventrally directed slender process.

Description.

Length of male, 4.3 mm.

Coloration. Crown and pronotum with dull orange and black scattered markings. Antennae with pedicel and flagellum light brown, scape dull yellow. Frontoclypeus yellow with reticulated black pattern, median black stripe indistinct. Clypeogenal, laterofrontal suture, gena, lorum with black markings. Clyepllus with median orange marking, bordered laterally by black marking. Mesonotum with black basal triangle bordered by dull orange marking, median indistinct light orange and black markings. Scutellum with pair of dull orange stripes. Legs yellow, fore-and hind femur with transverse black bands near apex and base, mid femur with transverse black band near base. Hind femur with black line along anterior margin.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein partially present. Metatibial setal rows PD, AD, and AV with 15, 9 and 11 macrosetae respectively.

Male genitalia. Pygofer broad with posterolateral margin continued as dorsal process and one subdorsal process arising basiventrally, both processes with truncate apex and directed slightly ventrad. Subgenital plate in lateral view slightly depressed basally, slightly broad near apex, long fine setae extending dorsal two thirds confined to lateral margin and dorsum of apex; in ventral view lateral margin gradually narrowed beyond middle near apex, widespaced submarginal macrosetae confined to apical half along lateral margin. Style in lateral view with basal apodeme broad; apophysis sinuate, broadened near middle, then abruptly narrowed and curved dorsad beyond middle, then slightly bent anterad with acute process near apex. Connective semicircular, fused to preatrium of aedeagus. Aedeagus with atrium narrow, socle poorly developed, shaft short and stout, in lateral view broad, sinuate, laterally flattened, inner lateral margin serrated, apex with pair of slender process arising inner lateral margin directed ventrad. Gonopore apical.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 15 – 22. XI. 2001, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-03 (CAS).

Etymology. The specific epithet is derived from the Latin words *brevi* meaning short and *pinguis* meaning stout, referring to the unique shape of the aedeagus.

18. *N. nanus,* sp. nov.

Fig.5. 4O, P; Fig.5. 11A–G

Diagnosis. This species is similar in habitus to *N. unimaculatus*, but may be distinguished by the following combination of characters: smaller body size; pygofer with posterodorsal process forked; style short with abruptly acuminate apex and aedeagus with two pairs of ventrally directed processs; one pair short, straight and the other long, curved.

Description.

Length of male, 3.25 mm.

Coloration. Crown with pair of median black markings not extending to face. Antennae, face, legs and forewings unmarked. Pronotum with central large elongate black spot. Mesonotum with basal black triangle extending to scutellum, median pair of black diverging lines not extending beyond scutellar suture. Forewing mostly hayaline.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 11, 8 and 8 macrosetae respectively.

Male genitalia. Pygofer broad with falcate posterodorsal process, process in posterior view bifurcate at the apex, with both branches equal in length; posterolateral margin with long setae. Subgenital plate in lateral view depressed basally, broad near apex, long fine setae confined to lateral margin and dorsum of apex; in ventral view lateral margin strongly convex, narrowed towards apex, with two rows of lateral and single row of mesal submarginal macrosetae. Style in lateral view with broad apodeme; apophysis abruptly tapered beyond midlength, apex acuminate, curved posterodorsad; in posterior view not reaching apex of aedeagal shaft. Connective in dorsal view semicircular, fused to preatrium of aedeagus. Aedeagus in lateral view with narrow, socle region well developed, shaft in lateral view slender, long, broadly curved anterodorsad with a pair of short processes arising on lateral surface of shaft; shaft in posterior view narrow, with pair of long ventrolaterally directed processes (broken in type specimen), slightly broad near middle. Gonopore subapical on posterior surface of the shaft

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 30. X. – 6. XI. 2005, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-128 (CAS).

Etymology. The specific epithet is derived from the Latin word *nanus* meaning small, referring to small size of the species.

19. *N. mananarensis*, sp. nov.

Fig.5. 4Q, R; Fig.5. 11H–N

Diagnosis. This species is similar to *N. orbiculatus* in habitus, the abruptly bent pygofer posterodorsal process, and the slightly convex mesal margin of the subgenital plate, but may be separated by the following combination of characters: subgenital plate with apex not curved dorsad; style with acuminate apex and aedeagus with pair of short curved apical processes.

Description.

Length of male, 4.3 mm.

Coloration as in *N. orbiculatus*.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 11, 8 and 8 macrosetae, respectively.

Male genitalia. Pygofer broad with pointed posterodorsal process, process in posterior view curved and pointed apex; posterolateral margin with short setae. Subgenital plate in lateral view depressed basally, with sinuate dorsal margin, rounded and slightly dorsally curved rounded apex; in ventral view lateral margin near base broadly convex, gradually tapering to apex, with biseriate macrosetae along lateral margin extending almost near base, a few macrosetae scattered near mesal margin and along middle near apex. Style in lateral view with broad apodeme; apophysis narrowed along middle, with acuminate apex, apex in posterior view not extending half length of shaft. Connective in dorsal view semicircular, fused with preatrium of aedeagus. Aedeagus in lateral view with narrow atrium, socle well developed, shaft slender, tubular, slightly narrowed towards apex; shaft in posterior view with pair of short terminal processes, extending laterally. Gonopore subapical basad of terminal process on posterior surface of shaft.

Materials examined. Holotype male, MADAGASCAR: Province Toamasina, Parc National Mananara-Nord, 7.1 km Antanambe, 225 m, 16°27’18” S, 49°47’15” E, 16. XI. 2005, Brian L. Fisher et al., on low vegetation rainforest, BLF12704 (CAS).

Etymology. The specific epithet refers to the type locality.

20. *N. quinquespinosus,* sp. nov.

Fig.5. 4S, T; Fig.5. 11O–U; Fig.5. 16I, J

Diagnosis. This species differs from other species of *Nesocerus* as follows: pygofer with basiventral process extending ventrad; aedeagus with five processes and subapical processes longer than apical processes. This species is similar to *N. trimaculatus* in having five aedeagal processes but differs in their relative lengths and in the presence of a basiventral pygofer process.

Description

Length of male, 5.9–6.6 mm; female, 6.1–7 mm.

Coloration. Crown with pair of median transverse black stripe near anterior margin, pair of lateral round black spots and median transverse patch near posterior margin. Antennae with pedicel dull black, scape and flagellum light yellow. Frontoclypeus with median narrow discontinuous; clypeogenal sutures bordered by narrow black stripe. Gena without markings. Clypellus bordered by black marking. Pronotum with pair of large round black spots along anterior region, a median inverted T- shaped stripe, pair of small round black spots near posterolateral margin. Mesonotum with basal black triangle bordered by light marking, median diverging black lines not extending posterad of scutellar suture. Forewing with claval vein white with alternate brown coloration. Fore- and hind femur with black stripe along ventral and dorsal margin respectively.

Structure. Clypellus with sides concave, apex wider than base, in profile slightly convex. Rostrum not extending beyond mid coxae. Forewing with r-m_1 crossvein absent. Metatibial setal rows PD, AD, and AV with 18–20, 9–10 and 12 macrosetae respectively.

Male genitalia. Pygofer broad without posterodorsal process, with inner process having round apex and preapical tooth extending ventrad along posterolateral margin; posterolateral margin with short setae. Subgenital plate in lateral view weakly depressed; in ventral view lateral margin broadly convex, with macrosetae confined to lateral and mesal margin. Style in lateral view with narrow apodeme; apophysis slender, elongate, curved dorsad, tapering to dorsally hooked apex. Connective in dorsal view Y-shaped, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium broad; socle poorly developed; shaft in lateral view narrow, curved broadly anterad, slightly tapered near apex; apex in posterior view with pair of short terminal processes extended laterad, another pair of ventrolaterally directed subapical long processes, not extending beyond half length of shaft; single long, slender unpaired process arising basal to subapical

processes near right lateral margin of shaft, arched ventrally to left and extended to base of shaft; gonopore apical on posterior surface of shaft basad of terminal processes.

Female genitalia. Second valvulae in lateral aspect blade shaped, gradually narrowed to apex; apical third of dorsal margin with irregulary arranged projections; ducts conspicuous.

Materials examined: Holotype male, MADAGASCAR: Province Antsiranana, Reserve Spéciale de l'Ankarana, 22.9 km SW Anivorano Nord, 80 m, 12°54'32"S, 49°6'35" E, 10-16. II. 2001, B. Fisher, C. Griswold et al., BLF2857 (CAS). *Paratypes*: 1 male, 2 females, same data as holotype (CAS, INHS); 1 male, MADAGASCAR: Province Antsiranana, Reserve Spéciale d'Ambre, 3.5 km, 235° SW Sakaramy, 325 m, 12°28'8" S, 49°14'32" E, 26-31. I. 2001, Fisher, Griswold et al., beating low vegetation, tropical dry forest, BLF2660 (INHS); 1 female, MADAGASCAR: Province d'Antsiranana, Reserve Spéciale d'Ambre, 3.5 km, 235° SW Sakaramy, 325 m, 12°28'8" S, 49°14'32" E, 26-31.I. 2001, Fisher, Griswold et al., malaise trap in tropical dry forest, BLF2661 (CAS).

Etymology. The specific epithet is derived from the Latin words *quinque,* meaning five and *spinosus,* meaning thorny, referring to the five processes of the aedeagus.

21. *N. asymmetricus,* sp. nov.

Fig.5. 5A, B; Fig.5. 12A–G

Diagnosis. This species can be distinguished by the following characters: aedeagus with apical and subapical process arising on anterior and posterior surface of shaft, respectively; pygofer with acute process extending mesad and crown with four black spots.

Description.

Length of male, 5.6 mm.

Coloration. Head with 4 submedian spots, 2 closer to posterior margin of crown and visible in dorsal view. Antennae with pedicel black, scape and flagellum yellow. Frontoclypeus with median longitudinal black stripe discontinuous or absent; clypeogenal sutures not marked. Gena without any markings. Clypellus bordered by narrow black stripe except along apex, with median yellow region. Pronotum with pair of black spots along anterior region, rarely, with median longitudinal narrow stripe, pair of small round spots near posterolateral margin. Mesonotum with pair of basal black triangles bordered with light marking, without median

markings. Scutellum without markings. Forewing with claval vein white with alternate brown coloration. Femora without markings.

Structure. Clypellus with sides concave, apex wider than base, in profile flat. Rostrum not extending beyond mid coxae. Forewing without r-m_1 crossvein. Metatibial setal rows PD, AD, and AV with 12, 8 and 9 macrosetae respectively.

Male genitalia. Pygofer elongate posterad, with a short inner process; posterolateral margin with short setae. Subgenital plates in lateral view spatulate; in ventral view with length approximately 3X width, lateral margin near base broadly convex gradually tapering to apex, macrosetae confined to apical half along lateral and mesal margins. Style in lateral view with narrow apodeme; apophysis slender, elongate, curved dorsad, tapering to dorsally hooked apex. Connective in dorsal view Y- shaped, with short stem, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view slender, uniform in width along its length, slightly narrowed near apex, curved broadly anterad near base, with two processes; one terminal process arising from anterior surface of shaft, curved ventrad, the other subapical process arising on posterior surface, extending ventrad, not beyond midlength of shaft; shaft in posterior view slightly narrowed near apex; gonopore subapical on posterior surface.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15’3” S, 47°24’25” E, 21-24. XII. 2001, R. Harin’Hala, malaise, mixed tropical forest, MA-02-09B-08 (CAS). *Paratype*: 1 male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Vohipara at broken bridge, 1110 m, 21°13’34” S, 47°22’11” E, 2-10. I. 2002, R. Harin’Hala, malaise trap in high altitude rainforest, MA-02-09A-10 (INHS).

Etymology. The specific epithet is derived from Latin, referring to the asymmetric processes of the aedeagus.

22. *N. uniprocessus,* sp. nov.

Fig.5. 5C, D; Fig.5. 12H–N; Fig.5. 16K, L

Diagnosis. This species may be distinguished by the short acute pygofer process arising dorsally near the middle of the posterolateral margin; the slender, elongate style with hooked apex; the Y-

shaped connective articulated with the aedeagus and single elongate subbasal process arising near the midlength of the aedeagal shaft and close to shaft throughout its length.

Description.

Length of male, 6 mm; female, 7 mm.

Coloration. Crown with pair of black spots lateral to median transverse black marking. Antennae with pedicel black, scape and flagellum light brown. Frontoclypeus with pair of oblique black stripe below ocelli extending to antennal ledge; median longitudinal black stripe narrow. Clypeogenal suture, gena umarked. Clypellus without any marking or black marking confined to apex and base. Pronotum with a few median tranverse black markings along anterior region, pair of large transverse black stripe close to posterior margin separated medially or several black spots close to posterior margin. Mesonotum with pair of yellow basal triangle, pair of median black line not extending basad of scutellar suture. Scutellum, forewing, and legs unmarked.

Structure. Clypellus with sides concave, apex as wide as base; in profile flat. Rostrum not extending beyond mid coxae. Forewing without r-m_1 crossvein. Metatibial setal rows PD, AD, and AV with 17–23, 9 and 11 macrosetae, respectively.

Male genitalia. Pygofer with short acute process arising dorsally near middle of posterolateral margin; posterolateral margin with short setae. Subgenital plates in lateral view weakly depressed; in ventral view lateral margin near base broadly convex gradually tapering to apex, short macrosetae confined to distal two thirds of lateral and mesal margins. Style in lateral view with narrow apodeme; apophysis slightly convex near dorsal margin, tapered beyond midlength to dorsally directed apex; in posterior view apex not extending half length of aedeagal shaft. Connective in dorsal view Y- shaped, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view slender, tubular, narrowed towards bifid apex, single subbasal process elongate arising near shaft midlength, close to shaft throughout length, extended to shaft apex; shaft in posterior view acuminate; gonopore apical on posterior surface.

Female genitalia. Second valvulae in lateral aspect blade shaped, preapical ventral margin slightly widened, then narrowed to apex; apical third of dorsal margin with widely spaced teeth not extending to extreme apex; ducts conspicuous.

Materials examined. Holotype male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15'3" S, 47°24'25" E, 21-24. XII. 2001, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-08 (CAS). *Paratypes*: 2 males, 1 female, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 22°15'3" S, 47°24'25" E, 28.I. – 4.II. 2002, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-14 (CAS, INHS).

Etymology. The specific epithet refers to the single process of the aedeagus.

23. *N. compressus,* sp. nov.

Fig.5. 5E, F; Fig.5. 12O–U

Diagnosis. This species may be distinguished by the following combination of characters: pronotum with single pair of black spots; pygofer with elongate poaterodorsal process; subgenital plate with uniseriate median row of macrosetae extending beyond its apical half and aedeagus laterally compressed with broad apex and single subbasal spine.

Description.

Length of male, 4.7 mm.

Coloration. Crown with median and two lateral black spots on posterior margin near eye. Antennae pale yellow. Frontoclypeus without median stripe, mostly pale yellow with a few black spots above ocelli. Clypeogenal suture bordered by black marking. Gena without nay marking. Clypellus mostly dull yellow with black lines near the base. Pronotum with pair of black spots. Mesonotum with yellow basal triangles and pair of black longitudinal lines not extending beyond scutellar suture. Legs pale yellow without any markings. Forewing hyaline with black coloration along claval margin.

Structure. Clypellus with sides concave, apex wider than base, in profile flat. Rostrum not extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 16, 7 and 9 macrosetae respectively.

Male genitalia. Pygofer moderately broad with elongate posterodorsal process; posterolateral margin with short setae. Subgenital plates in lateral view uniformly wide throughout length; in ventral view with lateral margin near base slightly convex, gradually tapering to apex, one row of macrosetae along middle. Style in lateral view with narrow apodeme; apophysis slender, elongate, curved dorsad, tapering to dorsally hooked apex. Connective in dorsal view Y- shaped,

with short stem and slender anterior arms, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium broad; socle poorly developed; shaft in lateral view broad, uniformly wide throughout its length, laterally compressed, slightly curved anterodorsad, broadly rounded apex, with spine arising near base; apex in posterior view without any processes; gonopore apical on posterior surface.

Materials examined. Holotype male, MADAGASCAR: Province Mahajanga, Parc National Tsingy de Bemaraha, 3.4 km 93° E Bekopaka, Tombeau Vazimba, 50 m, 9°8’31” S, 44°49’41” E, 6-10. XI. 2001, Fisher, Griswold et al., malaise trap, in tropical dry forest, BLF4233 (CAS).

Etymology. The specific epithet refers to the laterally compressed aedeagus.

24. *N. flexus,* sp. nov.

Fig.5. 5G, H; Fig.5. 13A–G

Diagnosis. This species may be distinguished by the following combinations of characters: lorum with median black marking; pygofer elongate and tapered posterad and aedeagal shaft strongly bend near middle, with short apical processes arising both anterior and posterior surfaces.

Description.

Length of male, 4.7 mm.

Coloration. Crown with black spot near compound eye, number of median irregular spots extending to face. Frontoclypeus with discontinuous median black stripe. Clypeogenal and laterofrontal suture broaded by black marking. Gena with tiny black spot below eye. Lorum with median black marking. Clypellus pale yellow with black line along lateral margins. Pronotum with median black line not extending to posterior margin, pair of lateral black spots and a few black spots near lateral margin. Mesonotum with pale yellow basal triangle, black marking near base, median inverted v-shaped black marking not extending beyond scutellar suture. Scutellum with two median black spots along lateral margin. Forewing hyaline with intermittent brown and white coloration. Legs pale yellow with black bands near apex. Fore femur with additional black marking near base.

Structure. Clypellus with sides slightly concave, apex wider than base, in profile flat. Rostrum not extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 18, 9 and 9 macrosetae respectively.

Male genitalia. Pygofer elongate, tapered posterad, without posterodorsal process; posterolateral margin with short setae. Subgenital plate in lateral view not depressed, broad near base, then gradually tapered to apex; in ventral view outer lateral margin slightly convex near base, with macrosetae confined to distal two thirds of lateral and mesal margins. Style in lateral view with narrow apodeme; apophysis slender, elongate, curved dorsad, tapering to dorsally hooked apex. Connective in dorsal view Y- shaped with short stem and widely diverging anterior arms, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium broad; socle reduced; shaft in lateral view broad, strongly bend anterad near middle, then abruptly tapered posterad, with single anterior and posterior process arising on either side of apex; apex in posterior view pointed, expanded subapically, with base of posterior process T-shaped with bulbous stem; gonopore subapical on posterior surface of shaft basad of terminal process.

Materials examined. Holotype male, MADAGASCAR: Province Mahajanga, Parc National Tsingy de Bemaraha, 3.4 km 93° E Bekopaka, Tombeau Vazimba, 50 m, 19°8’31” S, 44°49’41” E, 6-10. XI. 2001, Fisher, Griswold et al., malaise trap, in tropical dry forest, BLF4233 (CAS).

Etymology. The specific epithet is derived from the Latin root-word *flexi* meaning to bend, referring to the unique shape of the aedeagal shaft.

25. *N. breviprocessus,* sp. nov.

Fig.5. 5I, J; Fig.5. 13H–N

Diagnosis. This species may be distinguished from other species of *Nesocerus* by the following combination of characters: lorum with median black marking; pygofer with posterolateral margin almost truncate with short acute process arising near base; style elongate, slightly sinuate with flattened hooked apex; aedeagal shaft broad, elongate with short terminal sinuate processes and apical gonopore. This species is similar to *N. serratus* in the marking on the lorum, shape of the aedeagal shaft and position of the gonopore, but may be distinguished by the short aedeagal processes and the shape of the pygofer.

Description

Length of male, 4.3 mm.

Coloration. Crown with pair of lateral black spots and median black markings extending to face. Antennae dull yellow. Frontoclypeus without median black stripe, pair of lateral light brown stripe. Clypeogenal suture not bordered by black line. Laterofrontal sutre bordered with black

marking. Lorum with black marking. Gena with light brown marking below eye. Clypellus mostly light brown with yellow region near apex. Legs pale yeloow with pale brown colorations along surface of femora. Pronotum with discontinuous median black line, two pairs of black spots, one close to median line, the other near lateral margin. Mesonotum with broad basal black triangle separated medially by yellow spot not extending beyond scutellar suture. Forewing unmarked.

Structure. Clypellus with sides concave, apex as wide as base, in profile slightly convex. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein present. Hind femur missing.

Male genitalia. Pygofer broad, with short acute process arising posteriorly near base of truncate lateral margin; posterolateral margin with short setae. Subgenital plates in lateral view weakly depressed, uniformly wide throughout length, with short fine setae; in ventral view lateral margin near base broadly convex gradually tapering to apex, with macrosetae confined to distal two thirds of lateral and mesal margins. Style in lateral view with narrow apodeme; apophysis slightly convex near ventral margin, curved dorsad, uniformly wide throughout its length to anterodorsally directed hooked apex. Connective in dorsal view Y- shaped with short stem and narrowly diverging anterior arms, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view broad, straight, uniform in width throughout its length, slightly narrowed near apex; apex in posterior view rounded with pair of short terminal sinuate processes; gonopore apical, distad of apical processes.

Materials examined. Holotype male, MADAGASCAR: Tulear Province, Beza Mahafaly Reserve, Parcelle I near research station, 165 m, 23°41’11”S, 44°35’27” E, 4 – 11. XII. 2001, R. Harin’Hala, malaise trap in dry deciduous forest, MA-02-14A-05 (CAS).

Etymology. The specific epithet is derived from the Latin words *brevis*, meaning short, and *processus*, meaning process, referring to the short processes of the aedeagus.

26. *N. latus,* sp. nov.

Fig.5. 5K, L; Fig.5. 13O–U

Diagnosis. This species may be distinguished by the following unique characters: black transverse bands restricted to crown; pronotum and face without markings; pygofer with short sinuate acute process arising subdorsally on posterolateral margin; aedeagus with dorsolaterally

directed single forked subapical process and dorsally directed basal process and shaft apex in posterior view broad and rounded.

Description

Length of male, 4.3 mm.

Coloration. Crown with pair of rectangular shaped black markings with central yellow spot extending to ocellus. Antennae with scape and flagellum yellow; pedicel yellow. Frontoclypeus, gena, lorum, clypellus and legs without any black markings. Pronotum with oblique broad bands separated medially. Mesonotum with basal black triangle without nay median marking. Scutellum unmarked. Forewing hyaline with claval vein white, dark bands along anal veins.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 11, 8 and 8 macrosetae respectively.

Male genitalia. Pygofer broad, with short acute process arising near posteroventral margin; posterolateral margin with short setae. Subgenital plates in lateral view weakly depressed, lateral fold near base, with short fine setae; in ventral view lateral margin near base broadly convex gradually tapering towards apex, with macrosetae confined to distal two thirds of lateral and mesal margins. Style in lateral view with narrow apodeme; apophysis slender, elongate, tapered beyond midlength to dorsally directed hooked apex. Connective in dorsal view Y- shaped, stem and anterior arms short, stout, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium narrow; socle well developed; shaft in lateral view moderately broad, uniform in width throughout its length, straight, with dorsally directed long pair of processes arising near the base; apex in posterior view broadly rounded near apex, dorsolaterally directed long process arising subapically on posterior surface of shaft; gonopore subapical on posterior surface.

Materials examined. Holotype male, MADAGASCAR: Toamasina Province, 7 km SE of Andasibe National Park head- quarters, 1050 m, 18°57’45” S, 48°27’9” E, 23. III. – 7. IV. 2001, M. Irwin, R. Harin'Hala malaise trap, in tropical forest, MA-01-08A-04 (CAS).

Etymology. The specific epithet is derived from the Latin word *latus,* meaning broad, referring to the broad apex of the aedeagal shaft.

27. *N. basiprocessus,* sp. nov.

Fig.5. 5M, N; Fig.5. 14A–G

Diagnosis. This species may be distinguished by the following combination of characters: pygofer pointed posterodorsally with subdorsal broad process and aedeagus with single ventral and paired dorsal processes arising at base of shaft.

Description.

Length of male, 6.1 mm.

Coloration. Crown with two lateral and one median transverse black spots. Antennae with pedicel black, scape and flagellum light brown. Frontoclypeus with pair of black spots close to middle of eye. Laterofrontal suture bordered by black marking. Clypeogenal suture, gena, lorum without any markings. Clypellus mostly yellow with black marking along lateral margin and near apex. Pronotum with two black stripes; one median transverse band, other along posterior margin separated medially. Mesonotum with basal triangle orange, median spot near base, median longitudinal lines close to scutellar suture. Scutellum without markings.

Legs pale yellow, Fore-, mid- and hind femur with black marking near apex. Forewing mostly hyaline with claval vein white.

Structure. Clypellus with sides concave, apex as wide as base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 14, 10 and 10 macrosetae respectively.

Male genitalia. Pygofer broad with pair of pointed processes arising from posterolateral margin, dorsal one small and narrow, ventral one large and broad; posterolateral margin with short setae. Subgenital plate as in *N. uniprocessus*. Style in lateral view with narrow apodeme; apophysis slightly sinuate along dorsal margin, tapered beyond midlength to dorsally directed elongate apex; apex in posterior view reaching apex of shaft. Connective in dorsal view Y- shaped, articulated with preatrium of aedeagus. Aedeagus in lateral view with shaft broad, tubular, narrowly curved dorsad with two processes arising from base extending posterodorsad; ventral process single and dorsal process paired extending close to shaft; shaft in posterior view uniform in width throughout length. Gonopore apical on posterior surface.

Materials examined. Holotype male, MADAGASCAR: Province Toamasina, botanic garden near entrance to Andasibe National Park, 1025 m, 18°55’34” S 48°24’28” E, 15. VIII. – 1. IX. 2001, M. Irwin, R. Harin'Hala, malaise trap, tropical forest, MA-01-08B-10 (CAS).

Etymology. The specific epithet refers to the basal processes of the aedeagus.

28. *N. serratus*, sp. nov.

Fig.5. 5O, P; Fig.5. 14H–N

Diagnosis. This species is similar to *N. breviprocessus* in the shape of the aedeagal shaft, position of the gonopore and black marking on the lorum, but may be separated by the presence of long ventrolaterally directed aedeagal processes with serrated lateral margins and the crenulate lateral margin of the aedeagal shaft extending throughout its length.

Description.

Length of male, 3.9 mm.

Coloration. Crown with lateral elongate black spot close to eye margin; median pair of large black spots near posterior margin, pair of small median spots along anterior margin. Antennae completely dull yellow. Frontoclypeus without median stripe, light brown lateral markings along muscle impressions, Clypeogenal suture, laterofrontal suture, gena without any border; pair of oblique marking below laterofrontal suture. Lorum with central black spot. Clypellus mostly yellow with two light brown spot near base. Pronotum with four circular black spots; one median pair, close to median stripe, other pairlateral close to posterior margin. Mesonotum with orange basal triangle bordered by black marking, and median pair of longitudinal black lines not extending beyond scutellar suture. Scutellum without any markings. Forewing with pale yellow claval vein. Legs pale yellow without any markings.

Structure. Clypellus with sides concave, apex wider than base, in profile slightly convex. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 16, 9 and 8 macrosetae respectively.

Male genitalia. Pygofer with short process directed laterally in caudal view, posterolateral margin with short setae. Subgenital plate in lateral view depressed basally, broadened near apex with short fine setae confined to lateral margin and dorsum of apex; in ventral view lateral margin gradually convex towards base, abruptly narrowed beyond middle near apex, with mesal row of macosetae along distal two thirds. Style in lateral view with broad apodeme; apophysis elongate and uniformly wide throughout length, curved dorsad beyond middle with acuminate apex. Connective in lateral view V- shaped, articulated to preatrium of aedeagus. Aedeagus in lateral view with preatrium broad, socle well developed, shaft in lateral view broad, straight, laterally flattened; apex in posterior view narrow, with pair of long ventrolaterally directed

processes with serrated lateral margin. Shaft in posterior view with crenulate lateral margin extending from base to subapex. Gonopore apical.

Materials examined. Holotype male, MADAGASCAR: Tulear Province, Beza Mahafaly Reserve, Parcelle II near Bellevue, 180 m, 23°41’23” S, 44°34’31” E, 2 – 9. I. 2002, M. Irwin, R. Harin'Hala, malaise trap in spiny forest, MA-02-14B-09 (CAS).

Etymology. The specific epithet refers to the serrated margin of the aedeagal processes.

29. *N. convolutus,* sp. nov.

Fig.5. 5Q, R; Fig.5. 14O–U

Diagnosis.

This species may be distinguished by the following unique characters: presence of median brown band along costa of forewing; pygofer with short process directed posterad along posterolateral margin and aedeagal shaft twisted near apex with single short process, in caudal view with lateral flaps extending from base to subapex and pair of dentate apical process.

Description.

Length of male, 3.75 mm.

Coloration. Crown yellow with three black markings; median one V-shaped extending to face, lateral ones comma shaped with yellow spot at the apex. Antennae with pedicel and flagellum black, scape yellow. Frontoclypeus with short median black stripe not extending near base of clypellus. Laterofrontal suture and clypeogenal suture with black marking close to ocelli and base of clypellus, respectively. Gena with black elongate marking. Clypellus with U-shaped black marking. Lorum and legs yellow without any markings. Pronotum with numerous irregular black spots along middle and near lateral margins. Mesonotum with dull orange basal triangles, median longitudinal marking not extending beyond scutellar suture. Forewings mostly hyaline with dark brown veins, light brown band at middle along costal region.

Structure. Clypellus with sides concave, apex wider than base, in profile flat. Rostrum extending beyond mid coxae. Forewing with r-m1 crossvein absent. Metatibial setal rows PD, AD, and AV with 12, 10 and 10 macrosetae respectively.

Male genitalia. Pygofer broad with short process directed posterad in lateral view, posterolateral margin with short setae. Subgenital plate in lateral view slightly depressed basally, slightly broadened near apex, with short fine setae confined to dorsum of apex; in ventral view lateral

margin gradually narrowed beyond middle towards apex, with macrosetae along mesal and lateral margin. Style in lateral view with narrow apodeme; apophysis slender, uniform in width throughout length, curved dorsad near apex, apex hooked. Connective Y- shaped, articulated with preatrium of aedeagus. Aedeagus in lateral view with atrium broad, socle well developed, shaft in lateral view slender, twisted near apex, in caudal view with lateral flaps extending from base to subapex, gradually narrowed towards apex, with single lateral process arising near the bent, pair of short dentate processes near apex. Gonopore apical.

Materials examined. Holotype male, MADAGASCAR: Tulear Province, Forêt Ivohibe, 55.0 km N Tolagnaro, 200 m, 24˚34'08" S 47˚12'14" E, 2 – 4. XII. 2006, B.L.Fisher et al., malaise trap, rainforest, BLF15448 (CAS).

Etymology. The specific epithet refers to the unique twisted shape of aedeagus.

30. *N. trimaculatus* Freytag & Cwikla

Fig.5. 15 A–D

This species was described by Freytag & Cwikla (1984) based on a single male specimen from Ankarafantsika forest reserve in the Mahajanga province of Madagascar. We could not examine the holotype as it is apparently lost. We identified this species in present samples based on the similarity of both external morphology and genitalia characters. Pygofer, subgenital plate, style, connective and shape of aedeagus are as same as in original description. The only difference we noticed is in the number of aedeagal processes. Aedeagus in lateral view has 2 pairs of lateral apical processes and a single process arising on ventral surface of shaft subapically. In the original description, the presence of this single subapical process is not mentioned or illustrated and we assume that this process might have broken in the original specimen.

Materials examined. Six males, MADAGASCAR: Province Mahajanga: Parc National Tsingy de Bemaraha, 3.4 km 93° E Bekopaka, Tombeau Vazimba, 50 m, 19°8'31" S, 44°49'41" E, 6-10. XI. 2001, Fisher, Griswold et al., malaise trap, in tropical dry forest, BLF4233 (CAS, INHS).

31. *N. clavopunctatus* (Evans)

Fig.5. 1 E, F

Idiocerus clavopunctatus Evans 1953: 106

Nesocerus clavopunctatus (Evans) – Freytag & Knight 1966: 83

Comments. This species and *N. spurus* Freytag & Knight are very similar to each other, but different from other known *Nesocerus* species, in external color pattern (Fig.5. 1 E, F). Evans (1953) described *N. clavopunctatus* from a single female specimen based on external coloration and did not describe the genitalia. Freytag & Knight (1966) illustrated the female seventh sternum of *N. clavopunctatus* and described *N. spurus* based on a series of male and female specimens, distinguishing the latter species based on its smaller size and more truncate female seventh sternite. Comparison of the type and one non-type specimen of *N. clavopunctatus* to a series of specimens of *N. spurus* in the MNHN revealed that Freytag & Knight's (1966) illustration of the female seventh sternite of *N. clavopunctatus* is inaccurate and that its shape is very similar to that of *N. spurus*. Nevertheless, *N. clavopunctatus* differs from *N. spurus* as follows: body size larger, forewing with lateral yellow spot near base of clavus shorter and broader, median yellow spot on costal margin absent, and frontoclypeus with median black stripe. Although discovery of males from the type locality of *N. clavopunctatus*--Massif du Tsaratanana--may yet reveal that this taxon and *N. spurus* are color variants of the same species, the evidence available at present is sufficient to continue treating these two taxa as separate species.

Specimens of *N. spurus* examined. Six males, MADAGASCAR: Massif du Marojejy Andasy II, 1300m, IV. 1961, P. Soja (MNHN); holotype male, MADAGASCAR Est: district de Sambava, Réserve naturelle intégrale XII, massif du Marojejy-Ouest, 1140m, XI. 1959, P. Soga.

Specimens of *N. clavopunctatus* examined. Holotype female, MADAGASCAR Sambirano, massif du Tsaratanana, 1900m, X. 1949, R. Paulian; 1 female, MADAGASCAR nord, massif du Tsaratanana (versant Sud), 2030 m, Andohanambatoafa, 16-18. XII-1966, P. Soga (MNHN).

Phylogeny

Results and discussion

The phylogenetic analysis of 41 taxa and 65 characters resulted in 19662 equally parsimonious trees of 202 steps (CI = 0.5047; CI, for informative characters = 0.4904; RI = 0.7814; RC = 0.3944). Of 65 characters, 54 are parsimony informative. One of the most parsimonious trees is illustrated in Fig.5. 17. Some branches received moderate to high support values (Bremer support of 3 or higher), while most branches received support values of 2 or 1.

Monophyly of the genus *Nesocerus* is well supported with a decay index of 5 (node=9) in our analysis, supported by the following synapomorphies (the characters in Appendix 1 are referenced by the numbers given in parentheses): absence of pair of dorsoapical setae on first hind tarsomere (21), presence of longer lateral setae on pecten of first hind tarsomere (23), presence of three apical cells bordering appendix (25), presence of macrosetae on subgenital plate (38), absence of lateral lobe of style (51) and absence of short setae on style (54). Other notable synapomorphies include the presence of two longitudinal rows of setae on the first hind tarsomere (22), the dorsoventrally flattened subgenital plate (47) [also present in some outgroups], and the distally narrowed second apical cell of the forewing (29) [parallel in three species within *Nesocerus*].

Two moderately well supported clades, Clade A and Clade B within the genus were also identified, corresponding to groups of species that share distinctive morphologies.

Clade A comprises 19 species. The monophyly of this clade received moderately good branch support (node= 21, DI= 3) with unique synapomorphies including the U-shaped connective (57), fused connection of aedeagus with connective (59), and presence of two keels on the connective (60). Other synapomorphic characters are the position of the maxillary pit on a diagonal line from the clypellar suture (15) [except in three species within the genus] and the position of the gonopore basad of the aedeagal processes (64), except in *N. brevipinguis* where it is distad of the processes. Clade B comprises 12 species and was supported as a monophyletic group (node=10, D=1) by the well-developed lobe on the subgenital plate that is connected to the style (46), and the hooked style apex (50). Most of the species in this clade also have a uniseriate row of macroseate on the subgenital plate (41), short setae on the pygofer (33), a toothlike pygofer process (35), articulated aedeagus and connective (59), and narrow style apodeme (49), but these characters are present in some outgroups.

N. littoralis is unique, sharing morphological features with species of both clade A and B. It grouped with clade A based on the shape of the connective (57) and its fused connection with the aedeagus (59). Nevertheless, *N. littoralis* shares characters such as the toothlike pygofer process and short setae of the pygofer (33) with species in clade B.

In clade B, some nodes were moderately well supported. *N. recurvus* and *N. dorsalis* (node=36, DI=2) are supported as a monophyletic group mainly by two synapomorphies, the hooked apex of the pygofer process (37) and the style apex with ventral processes (50). *N.*

tulearensis and *N. nigrum* (node=29, DI=2) are supported as a monophyletic group mainly by two synapomorphies, the pygofer process with ventral process (35) and the truncate apex of the subgenital plate (43).

The relationship of *Nesocerus* to other genera of Idiocerinae is not well understood and the present results indicate that more characters and/or a larger sample of taxa will be needed to clarify this relationship. Although the tree in Fig.5. 17 places a clade of African taxa as sister to *Nesocerus* with no Bremer support, other most parsimonious trees showed different sister-group relationships. Among the 19662 equally parsimonious trees recovered by analysis of morphological data, 3836 had a clade comprising all taxa having three apical cells bordering the forewing appendix, a feature that is apparently restricted to *Nesocerus*, *Ceylonoscopus* from Sri Lanka, and two undescribed genera from Madagascar and Borneo. If this forewing character is a synapomorphy, then this suggests that *Nesocerus* is most closely related to taxa from South Asia, rather than Africa. Another morphological feature present in *Nesocerus,* but very rare in other Idiocerini, is the presence of a depressed subgenital plate. This feature is also present in many South American idiocerines, including the two included in this analysis and *Ceylonoscopus*. In the same trees that placed the clade comprising three South Asian species as sister to *Nesocerus*, this combined clade was sister to a clade comprising the two included genera from South America. This grouping was supported by the position of maxillary sensory pit near the ventral margin of the face (15), the absence of a third subapical cell in the forewing (27) and the depressed male subgenital plate (47). The South American genera also share with *Nesocerus* a semicircular connective.

These possible morphological synapomorphies were not sufficient in our analysis to provide unequivocal support for a particular arrangement of basal splits in the phylogeny, but nevertheless suggest some biogeographic scenarios contrary to the usual pattern reported for relationships of Malagasy to non-Malagasy animals. Evidence for physical connections between Madagascar, the Indian subcontinent and South America until the late Cretaceous have been hypothesized in higher vertrebrates (Evans, 2008). A broader phylogenetic analysis of Idiocerini, including additional genera from Africa, Asia, and South America may further elucidate the relationship of *Nesocerus* to non-Malagasy idiocerines. Additional Old World genera possessing the above mentioned synapomorphies have not been found in our studies of available specimens and previously published taxonomic studies. More thorough faunistic studies of the diverse but

largely undescribed faunas of both South America and mainland India, especially the Western Ghats-Srilankan biodiversity hotspot, are needed.

Although our analysis was unable to resolve the continental-scale biogeographic pattern unambiguously, it did reveal an interesting pattern within Madagascar itself, with the two main lineages of *Nesocerus* largely restricted to the eastern and western regions. Previous studies of other organisms including spiders, reptiles, and amphibians have recovered similar east-west sister lineages (Vences, 2004; Wood et al, 2007). In *Nesocerus*, most of the 38 species presently known are confined to the eastern humid forests of Madagascar and most of these species belong to clade A. Conversely, most species in clade B are found in the arid western part of Madagascar. Species belonging to clade B are apparently more plesiomorphic, based on their similarity in male genitalia characters to other idiocerines, particularly in the shape of the connective and its articulation with the aedeagus. Moreover, it is assumed that arid to seasonally dry biomes of Madagascar are older compared to humid to sub-humid biomes based on paleoclimatic history (Yoder & Nowak 2006). This suggests that the common ancestor of clade A dispersed from the dry region to the humid eastern forest and subsequently diversified. A test of this scenario will require more collecting to elucidate the distributions of species, and a more detailed phylogenetic analysis using molecular data to estimate divergence times.

Conclusion

Morphological evidence supports the monophyly of the genus *Nesocerus* and recovered two clades within the genus with moderately strong branch support. Relationships among species within these two main clades are poorly resolved due to uncertainities over homology of features of the highly diverse male genitalia. In the present analysis, all the species in clade B have the connective articulated to the aedeagus, features shared with other idiocerines and therefore apparently plesiomorphic. In contrast, species in clade A have the connective fused to the aedeagus and a U- shaped connective with two keels, features unique to this clade. Characters that are often conserved within idiocerine genera, such as the shape of the style apex, arrangement and shape of macrosetae and length of fine setae on the subgenital plate, length of setae on the pygofer, and shape of the pygofer are highly variable among the species of *Nesocerus*.

Because a large proportion of the *Nesocerus* species are known only from a few or single specimens, mostly males; intensive sampling will be needed prior to any attempt to resolve species level relationships using molecular data. Also, females of most of the described species are unknown and additional morphological characters, mainly of females and nymphs, may help to resolve relationships within genus. Due to the high degree of similarity in coloration and external morphology among species, females are difficult to associate with males for many species.

This study revealed that the endemic genus *Nesocerus* is much more speciose that was previously suspected. The known fauna has increased from 8 to 37 species. More species undoubtedly await discovery with more intense sampling in Madagascar. This study is the first to use phylogenetic methods in examining relationships within a genus of idiocerine leafhoppers. Additional knowledge of ecology and life history of these species may help to elucidate the factors that yielded the high degree of diversity in this group. This diverse endemic genus may be useful as a model organism for understanding diversification processes in Madagascar.

Figures and Tables

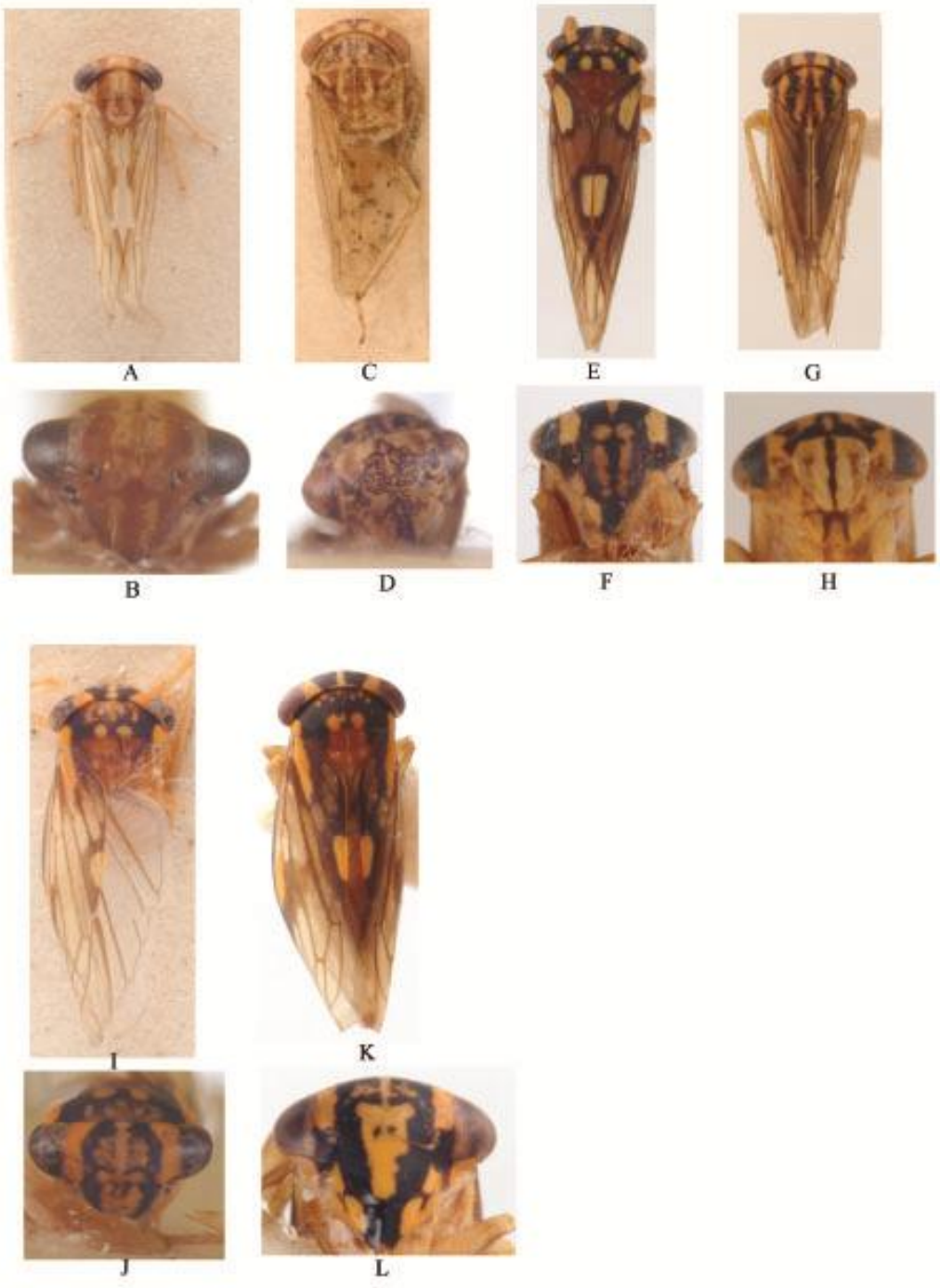

Figure 5.1 ***Nesocerus*** **spp., dorsal habitus & face. Holotypes, A-J; A, B,** ***N. acuminatus*** **Freytag & Knight.; C, D,** ***N.chelatus*** **Freytag & Knight; E, F,** ***N. clavopunctatus*** **(Evans); G, H,** ***N. madagascariensis*** **(Evans); I, J,** ***N.spurus*** **Freytag & Knight; K, L, female of** ***N.spurus*** **Freytag & Knight**

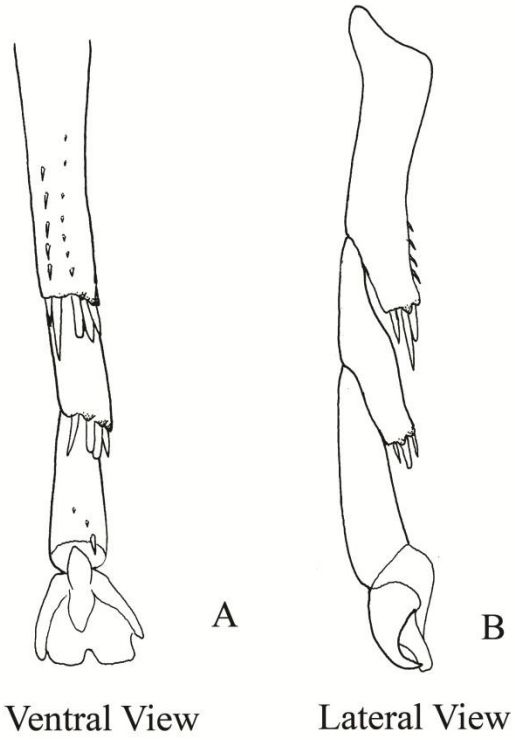

Figure 5.2 Hind tarsomere I. A, Ventral and B, Lateral view

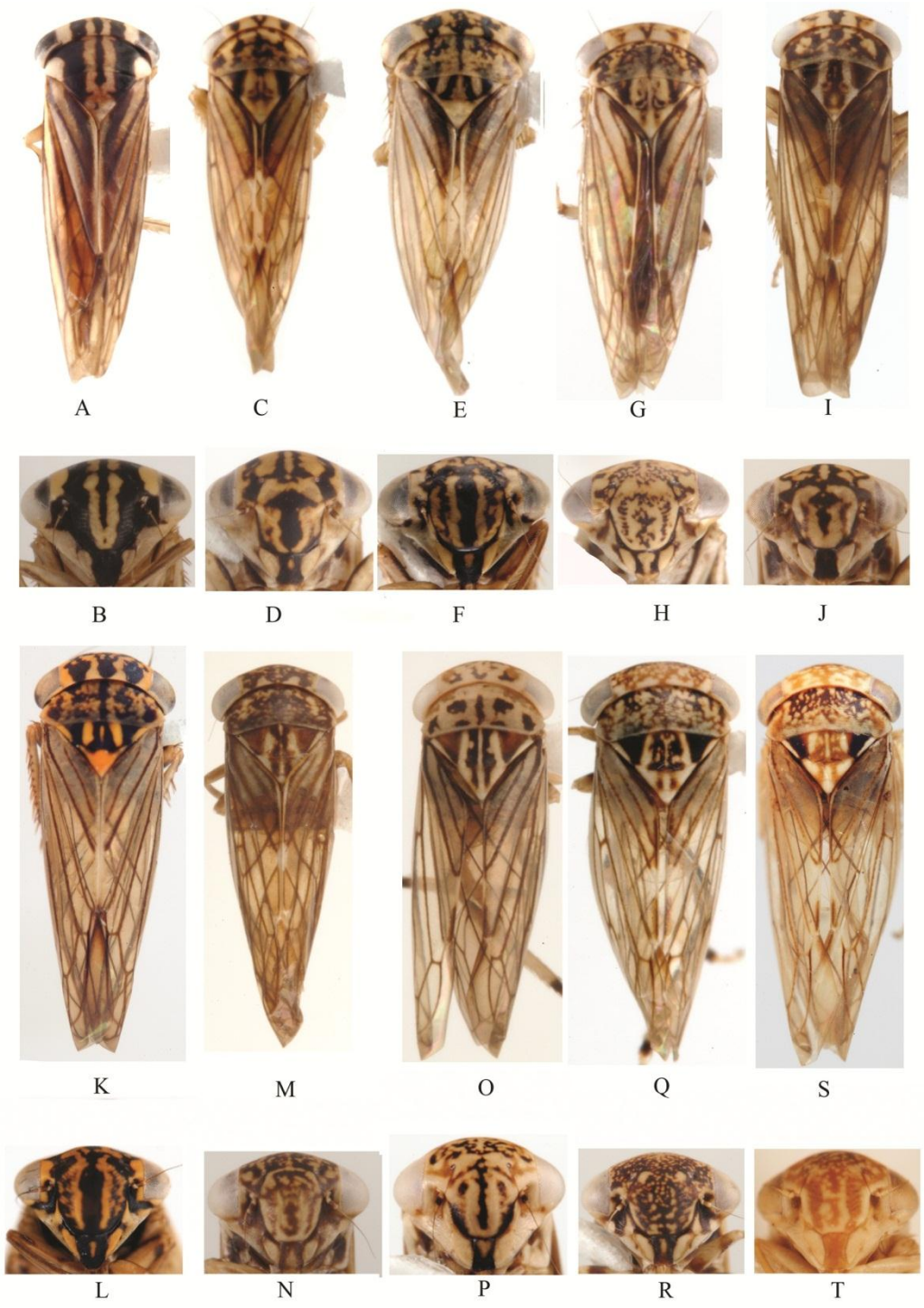

Figure 5.3 ***Nesocerus*** **new spp., dorsal habitus & face of holotype male. A, B,** ***N. trilineatus*****.; C, D,** ***N. ranomafanaensis;*** **E, F,** ***N. angulatus*****; G, H,** ***N. orbiculatus*****; I, J,** ***N. affinis*****; K, L,** ***N. sclerophyllus*****; M, N,** ***N. bifurcatus*****; O, P,** ***N. littoralis*****; Q, R,** ***N. fasika*****; S, T,** ***N. tulearensis***

Figure 5.4 ***Nesocerus*** **new spp., dorsal habitus & face of holotype male. A, B,** ***N. recurvus.*****; C, D,** ***N.dorsalis;*** **E, F,** ***N. dentatus*****; G, H,** ***N. unimaculatus*****; I, J,** ***N. nigrum*****; K, L,** ***N. tristriatus*****; M, N,** ***N. brevipinguis*****; O, P,** ***N. nanus*****; Q, R,** ***N. mananarensis*****; S, T,** ***N. quinquespinosus***

Figure 5.5 ***Nesocerus*** **new spp., dorsal habitus & face of holotype male. A, B,** ***N. asymmetricus*****; C, D,** ***N.uniprocessus;*** **E, F,** ***N. compressus*****; G, H,** ***N. flexus*****; I, J,** ***N. breviprocessus*****; K, L,** ***N. latus*****; M, N,** ***N. basiprocessus*****; O, P,** ***N. serratus*****; Q, R,** ***N. convolutus***

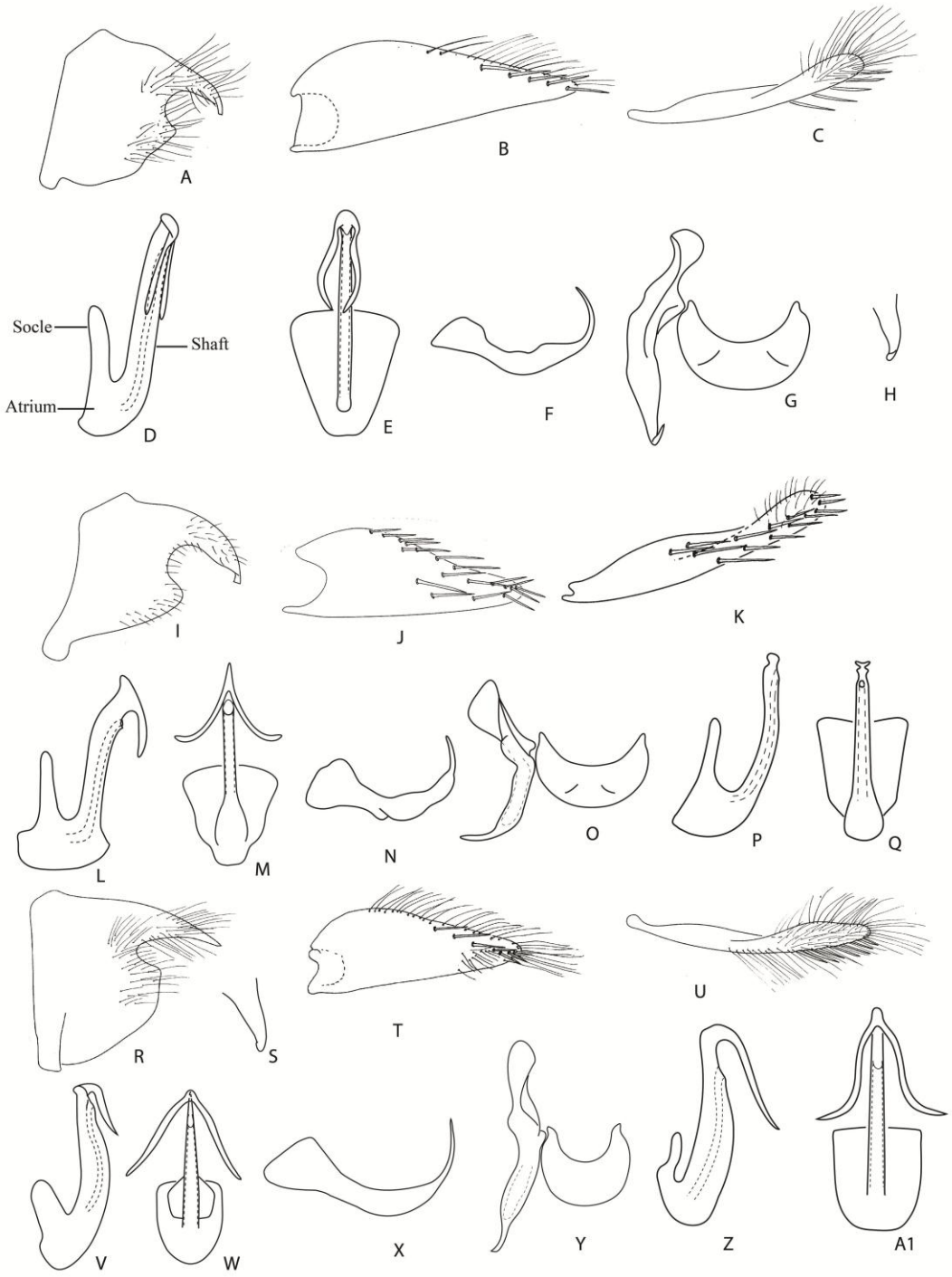

Figure 5.6 *Nesocerus* new spp. Male pygofer in lateral view (A, I, R), male subgenital plate in ventral view (B, J, T) and lateral views (C, K, U), aedeagus in lateral view (D, L, P, V, Z) and caudal (E, M, Q, W, A1) views, style in lateral view (F, N, X), style and connective in dorsal view (G, O, Y), apex of male pygofer in caudal view (H, S). A–H, *N. trilineatus*; I–O, *N. ranomafanaensis*; P–Q, *N. affinis*; R–Y, *N. angulatus*; Z–A1, *N. sclerophyllus*.

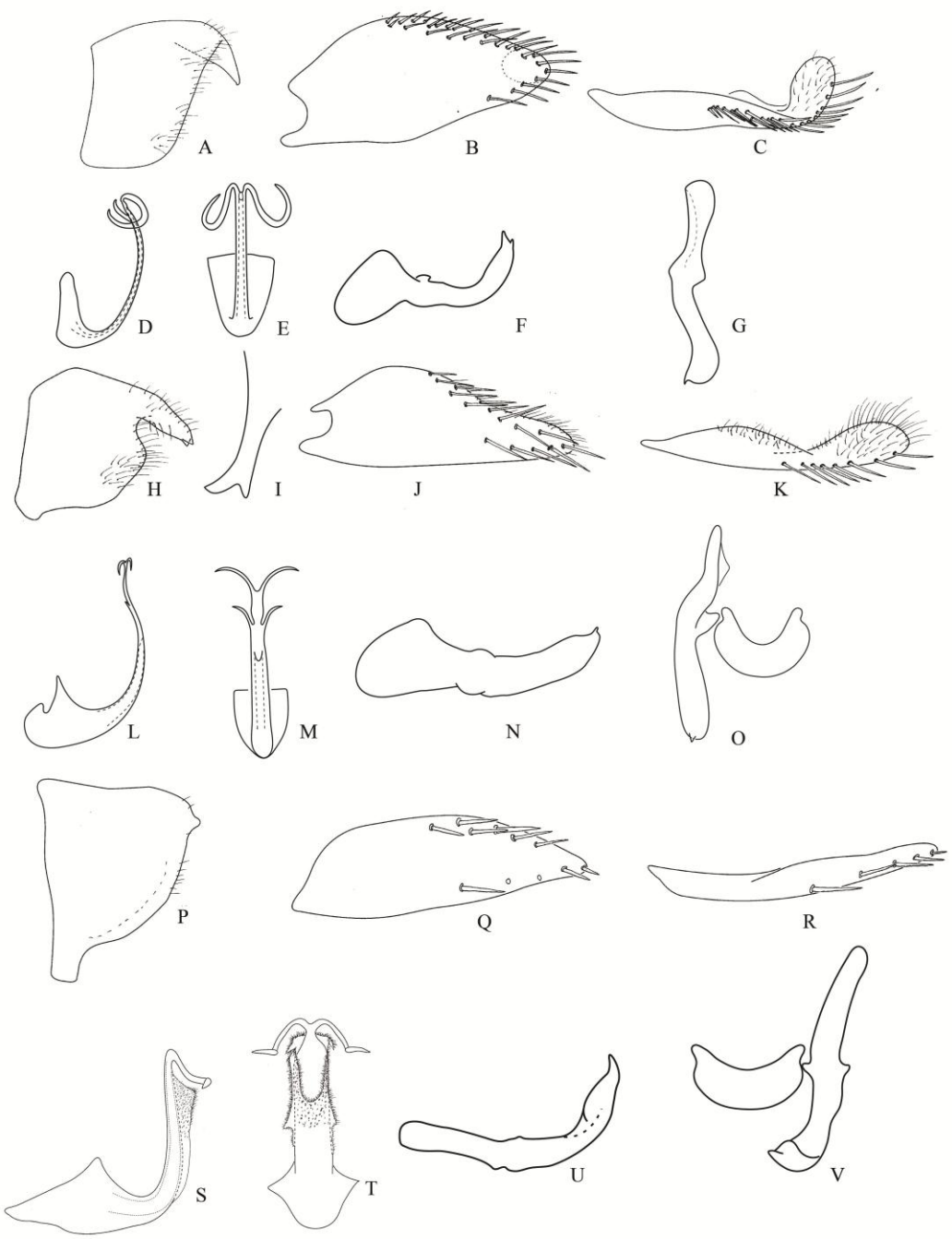

Figure 5.7 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, P), male subgenital plate in ventral view (B, J, Q) and lateral views (C, K, R), aedeagus in lateral view (D, L, S) and caudal (E, M, T) views, style in lateral view (F, N, U), style and connective in dorsal view (G, O, V), apex of male pygofer in caudal view (I). A–G,** ***N. orbiculatus*****; H–O,** ***N. bifurcatus*****; P–V,** ***N. littoralis*****.**

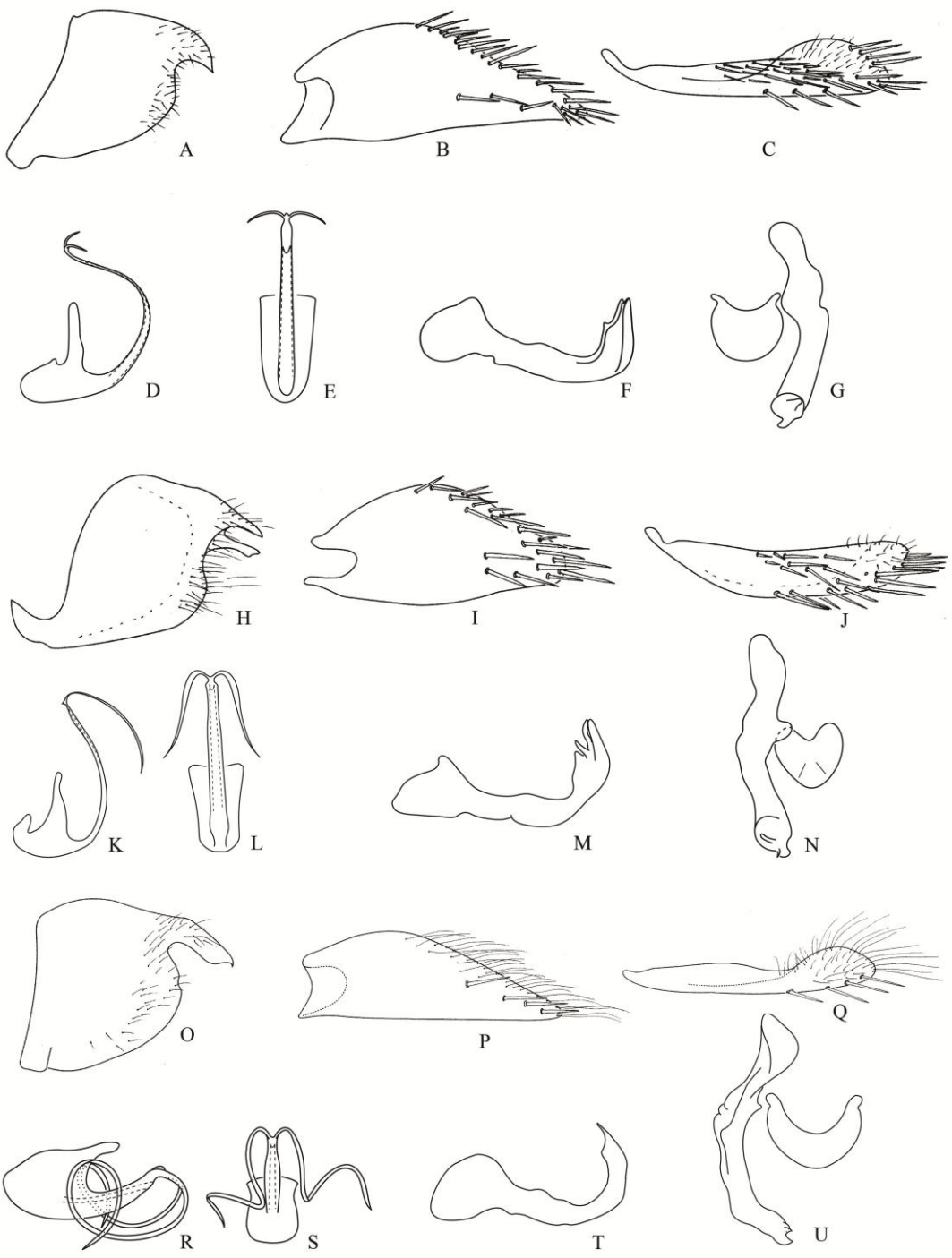

Figure 5.8 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, O), male subgenital plate in ventral view (B, I, P) and lateral views (C, J, Q), aedeagus in lateral view (D, K, R) and caudal (E, L, S) views, style in lateral view (F, M, T), style and connective in dorsal view (G, N, U). A–G,** ***N. fasika*****; H–N,** ***N. tulearensis*****; O–U,** ***N. recurvus*****.**

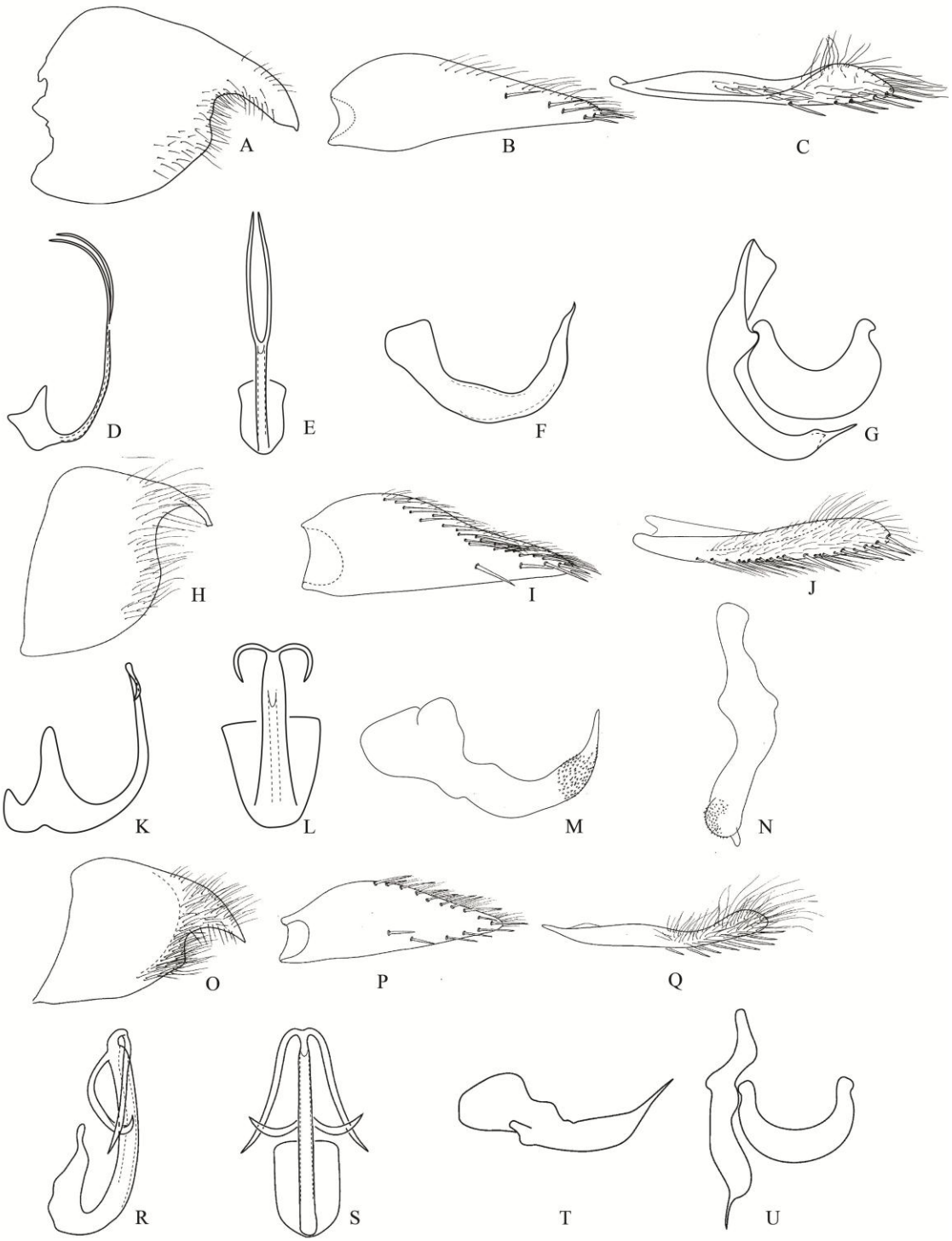

Figure 5.9 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, O), male subgenital plate in ventral view (B, I, P) and lateral views (C, J, Q), aedeagus in lateral view (D, K, R) and caudal (E, L, S) views, style in lateral view (F, M, T), style and connective in dorsal view (G, N, U). A–G,** ***N. dorsalis*****; H–N,** ***N. dentatus*****; O–U,** ***N. unimaculatus*****.**

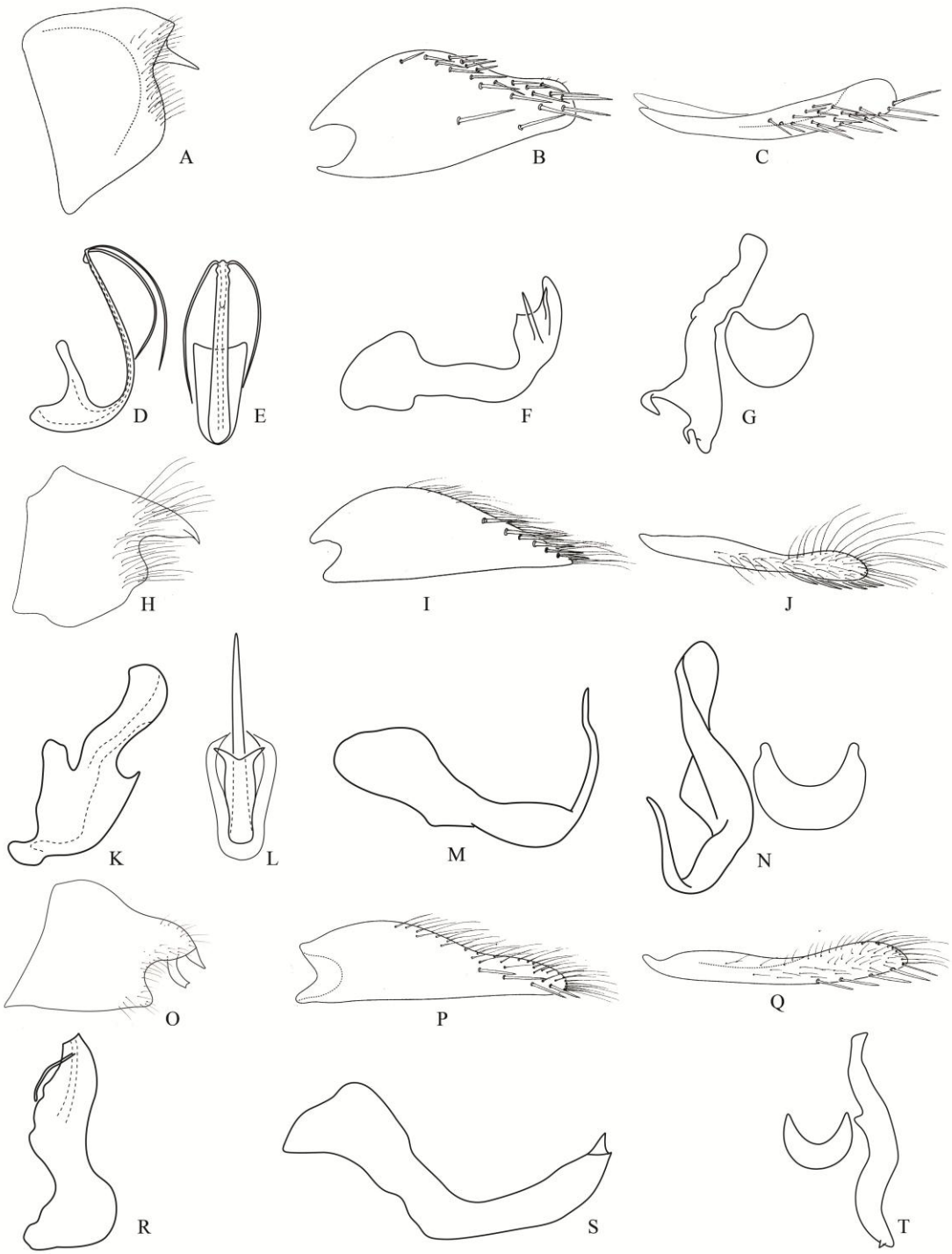

Figure 5.10 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, O), male subgenital plate in ventral view (B, I, P) and lateral views (C, J, Q), aedeagus in lateral view (D, K, R) and caudal (E, L) views, style in lateral view (F, M, S), style and connective in dorsal view (G, N, T). A–G,** ***N. nigrum*****; H–N,** ***N. tristriatus*****; O–T,** ***N. brevipinguis*****.**

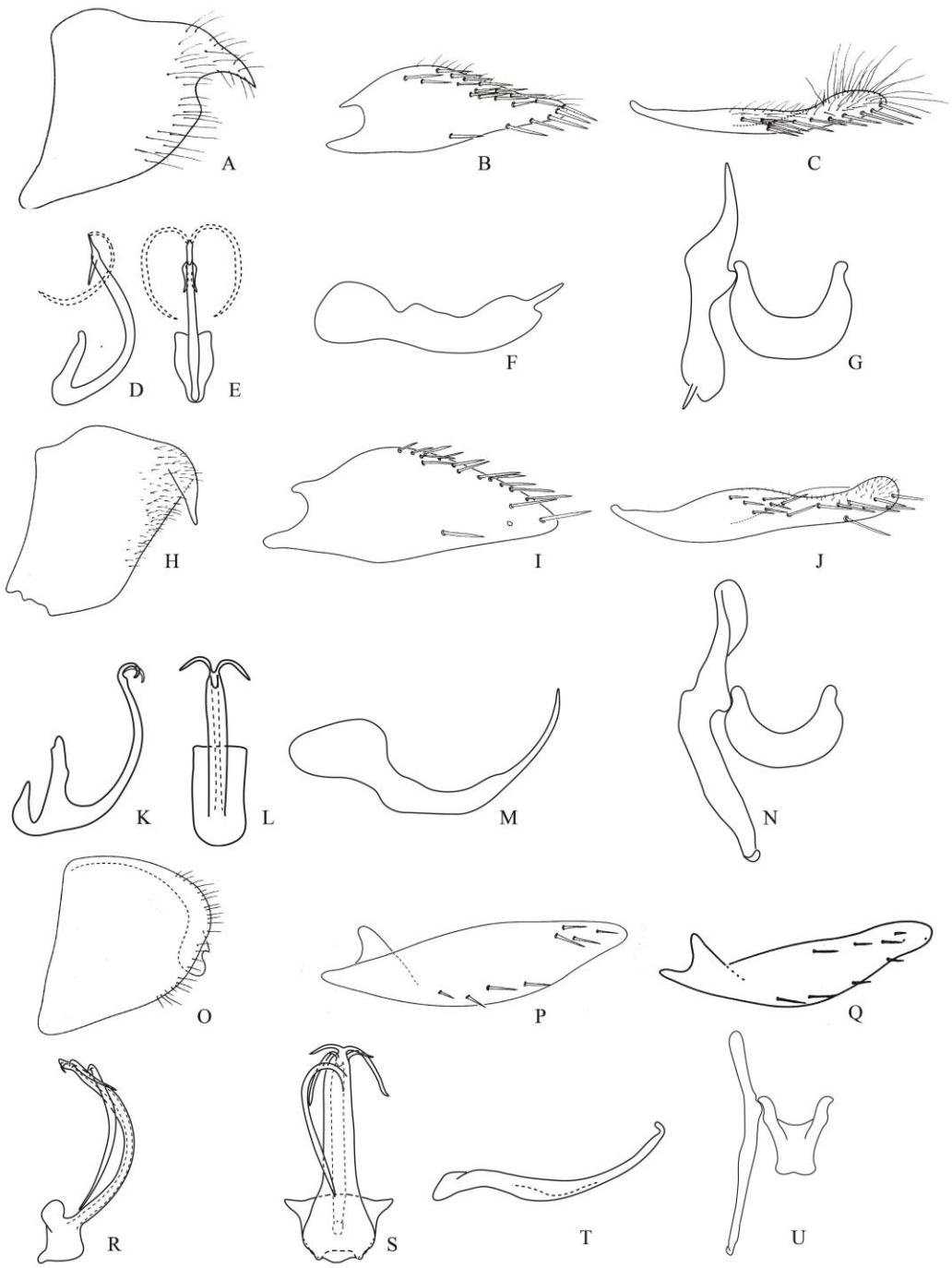

Figure 5.11 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, O), male subgenital plate in ventral view (B, I, P) and lateral views (C, J, Q), aedeagus in lateral view (D, K, R) and caudal (E, L, S) views, style in lateral view (F, M, T), style and connective in dorsal view (G, N, U). A–G,** ***N. nanus*****; H–N,** ***N. mananarensis*****; O–U,** ***N. quinquespinosus*****.**

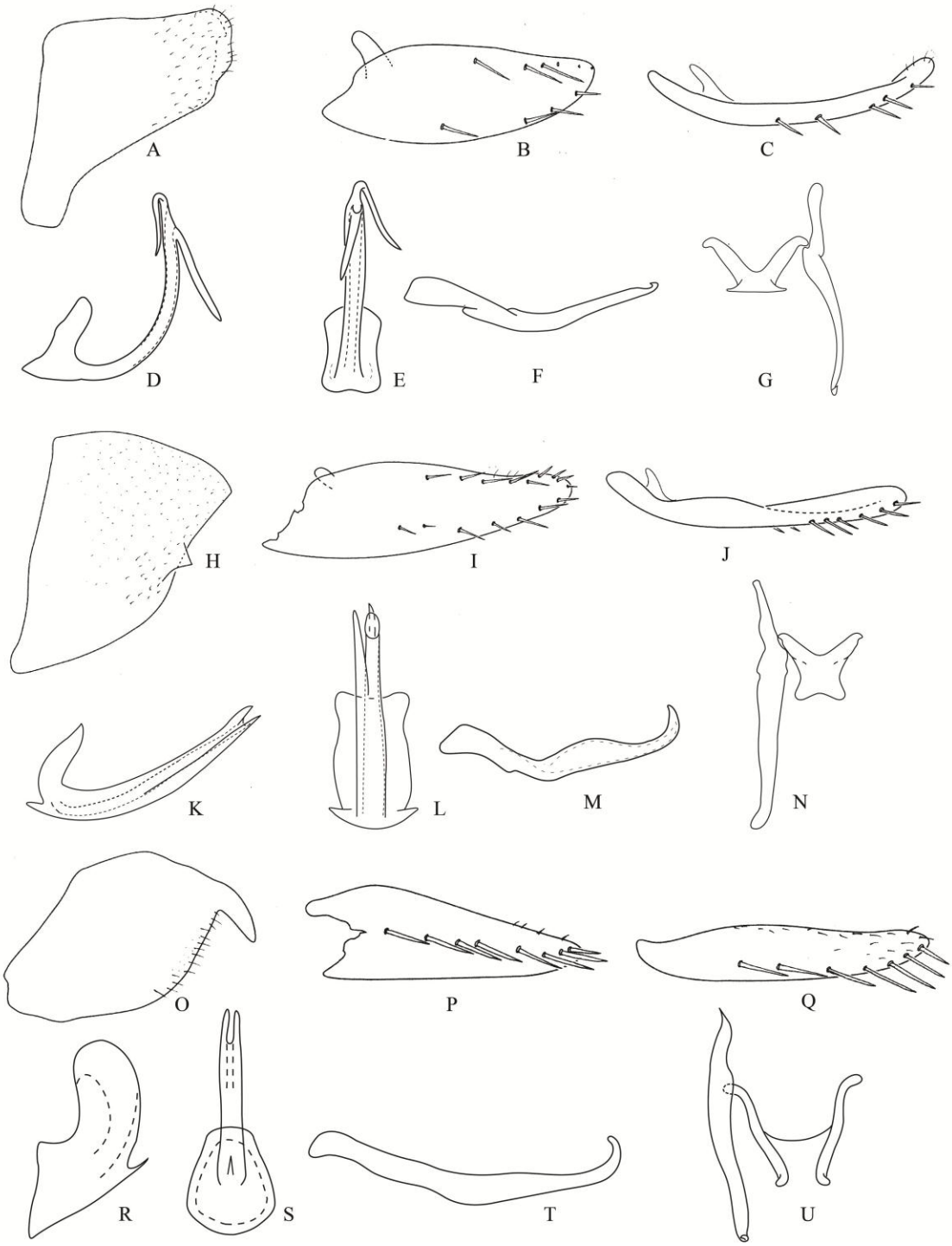

Figure 5.12 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, O), male subgenital plate in ventral view (B, I, P) and lateral views (C, J, Q), aedeagus in lateral view (D, K, R) and caudal (E, L, S) views, style in lateral view (F, M, T), style and connective in dorsal view (G, N, U). A–G,** ***N. asymmetricus*****; H–N,** ***N. uniprocessus*****; O–U,** ***N. compressus*****.**

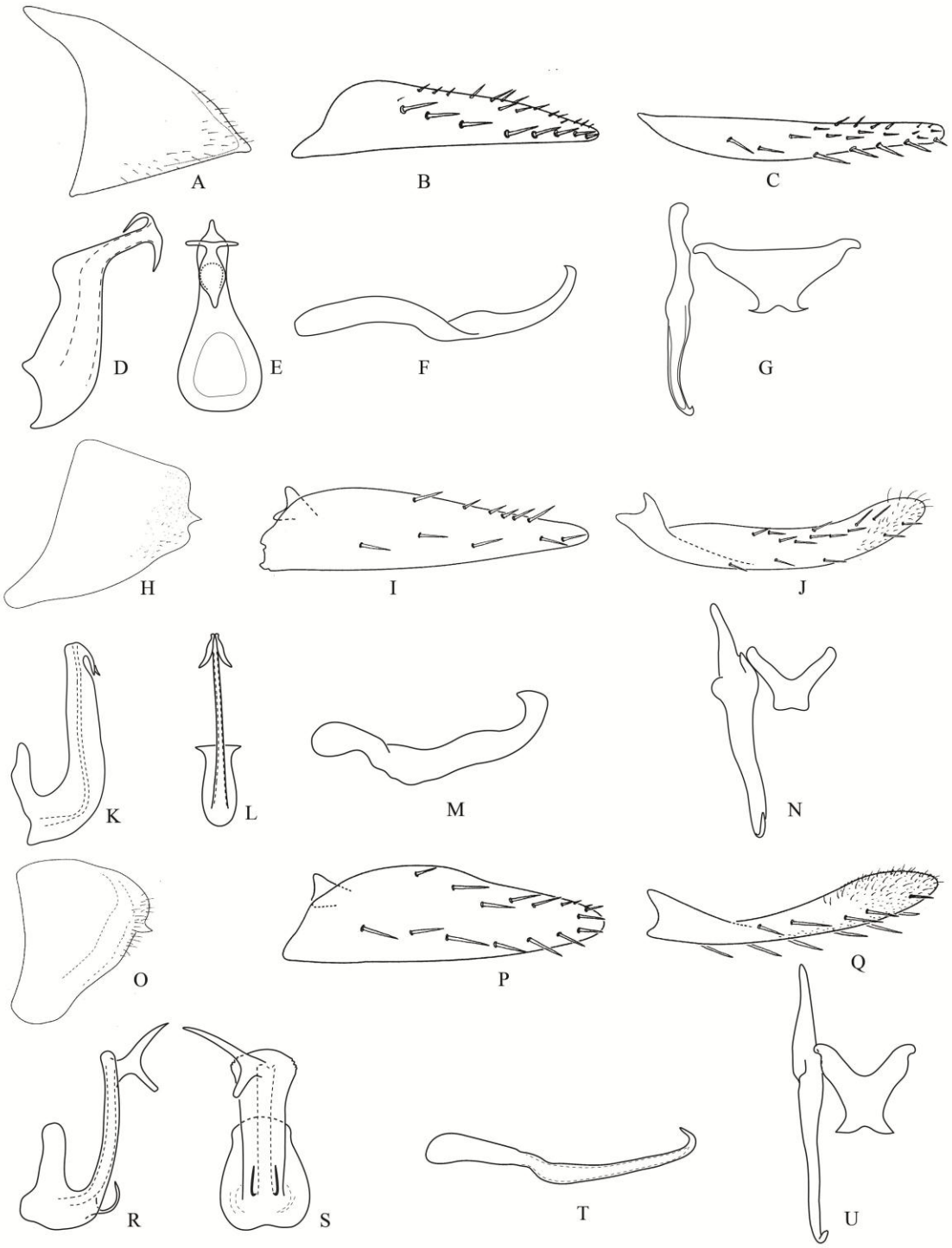

Figure 5.13 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, O), male subgenital plate in ventral view (B, I, P) and lateral views (C, J, Q), aedeagus in lateral view (D, K, R) and caudal (E, L, S) views, style in lateral view (F, M, T), style and connective in dorsal view (G, N, U). A–G,** ***N. flexus*****; H–N,** ***N. breviprocessus*****; O–U,** ***N. latus*****.**

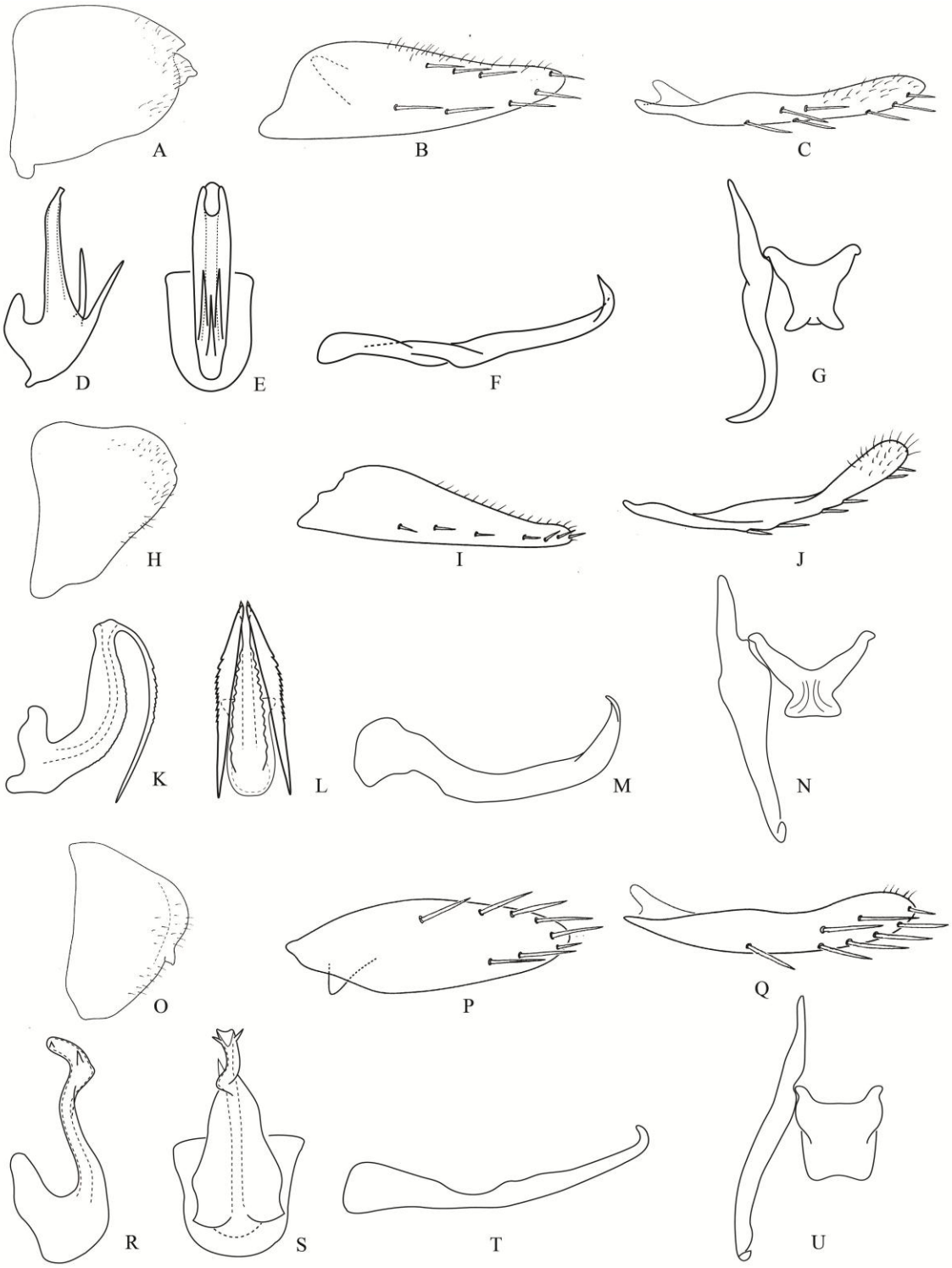

Figure 5.14 ***Nesocerus*** **new spp. Male pygofer in lateral view (A, H, O), male subgenital plate in ventral view (B, I, P) and lateral views (C, J, Q), aedeagus in lateral view (D, K, R) and caudal (E, L, S) views, style in lateral view (F, M, T), style and connective in dorsal view (G, N, U). A–G,** ***N. basiprocessus*****; H–N,** ***N. serratus*****; O–U,** ***N. convolutus*****.**

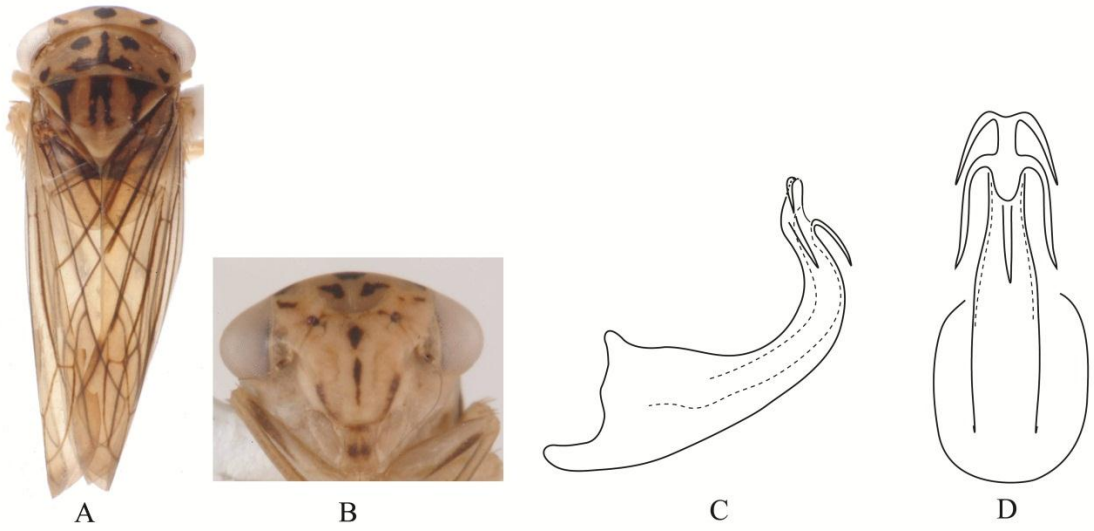

Figure 5.15 ***Nesocerus trimaculatus*** **Freytag & Cwikla.A, Dorsal habitus; B, Face; C–D, Aedeagus in lateral (C) and caudal (D) views.**

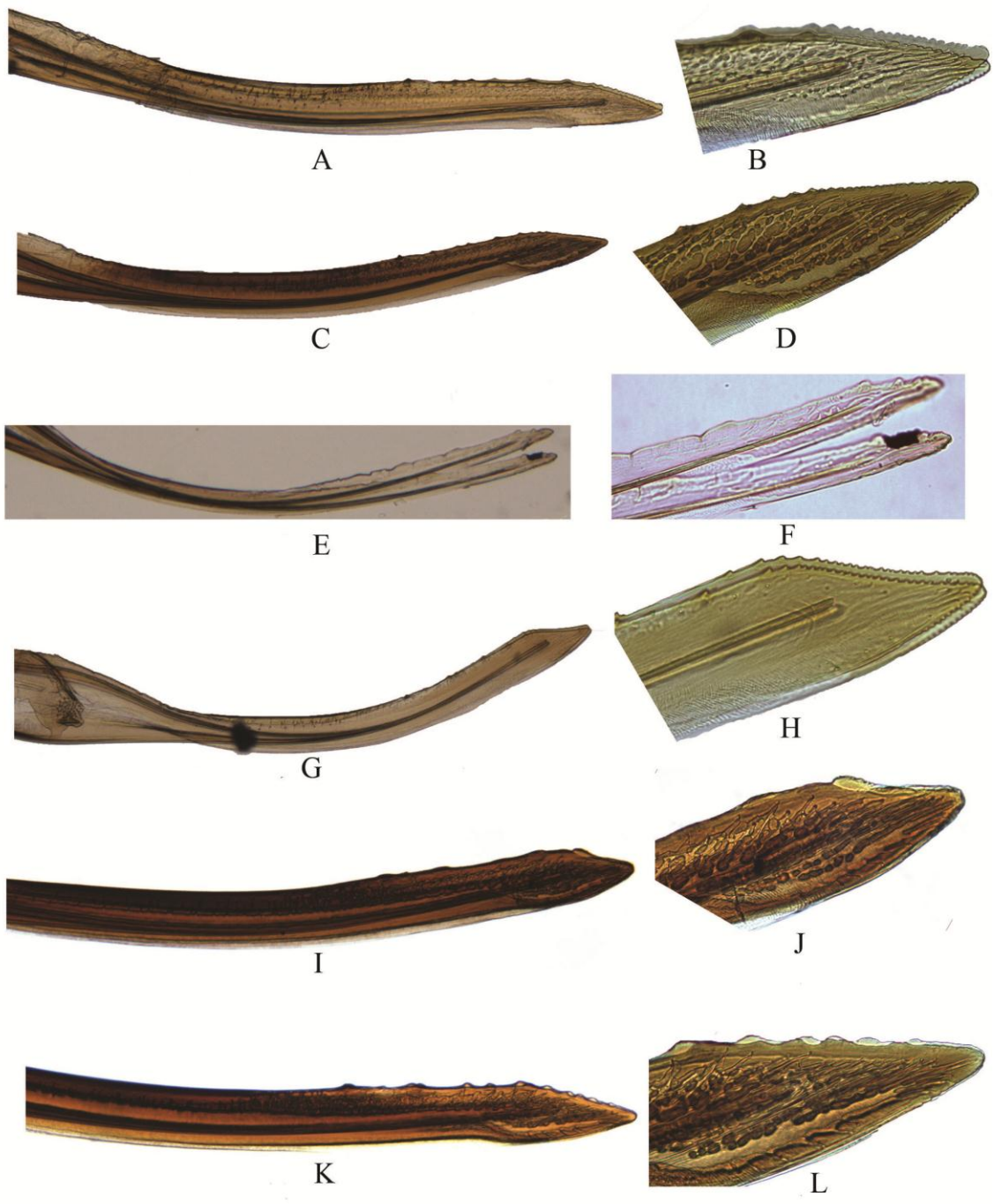

Figure 5.16 ***Nesocerus*** **spp., Second valvulae of female. left- lateral view, right- detail of sculpturing. A-B,** ***N. trilineatus*****; C-D,** ***N. sclerophyllus*****; E-F,** ***N. tulearensis*****; G-H,** ***N. dentatus*****; I-J,** ***N. quinquespinosus*****; K-L,** ***N. uniprocessus.***

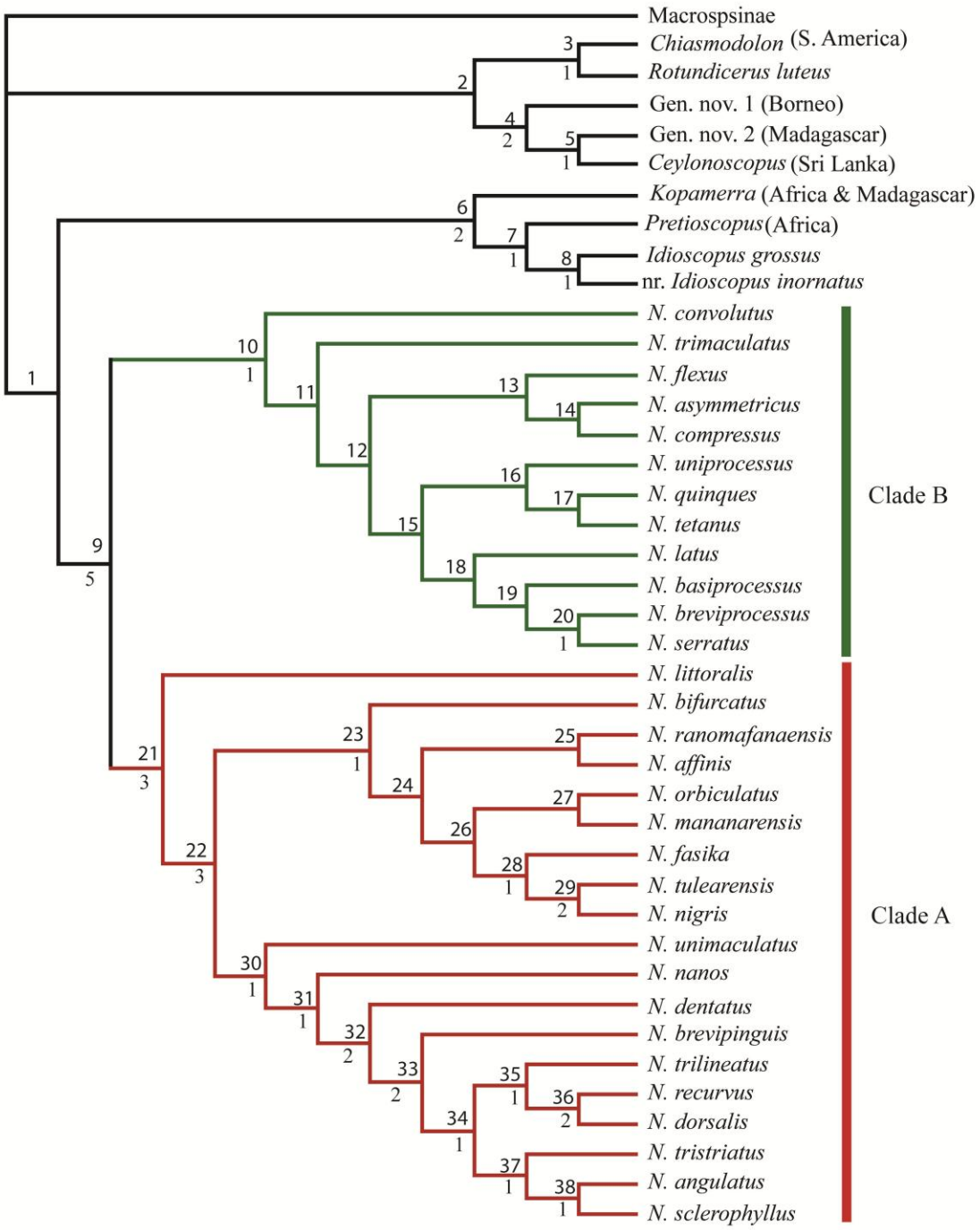

Figure 5.17 One of the 19662 equally most parsimonious trees of 202 steps (CI = 0.5047; CI, for informative characters = 0.4904; RI = 0.7814 RC = 0.3944). Numbers above branches are node numbers and those below are Bremer support values. Nodes without Bremer support were not recovered in all equally parsimonious trees.

Table 5.1 Morphological characters and states for phylogenetic anlaysis of *Nesocerus*

Head	
1	Microsculpture of head: 0, shagreened; 1, striate
2	Microculpture of pronotum: 0, shagreened; 1, striate
3	Presence of produced pronotum: 0, present; 1, absent
4	Markings on pronotum: 0, single spot 1, pair of spots along imaginary transverse line 2, mottled; 3, longitudinal bands; 4, absent
5	Lateral spot on vertex: 0, absent; 1, present
6	Laterofrontal suture: 0, present ; 1, absent
7	Laterofrontal suture: 0, reaching ocelli; 1, not reaching ocelli
8	Clypeogenal suture extending to antennal ledge: 0, extending ; 1, not extending
9	Setae on genal margin just ventrad of each eye: 0, absent; 1, present
10	Genae narrow: 0, narrow; 1, broad
11	Median width of lora: 0, same as midwidth of clypellus; 1, narrower; 2, broader
12	Lorum apex: 0, widely separated from genal margin; 1, not widely separated
13	Shape of lora:0, hemispherical; 1, almost straight (apex not well defined)
14	Median marking on lora: 0, present; 1, absent
15	Position of maxillary sensory pit: 0,slightly above diagonal line from clypellus suture to lora; 1, in diagonal from clypellus suture, below diagonal line from clypellus suture to lora; 3, at lowermost part of maxillary plate
16	Base of clypellus: 0, as broad as apex or preapex; 1, narrower than apex; 2, wider base
17	Posterior region of clypellus: 0, not depressed; 1, slightly depressed; 2, strongly depressed
18	Clypellar suture: 0, complete; 1, incomplete
Leg	
19	Hind setae formula: 0, 2+0; 1 2+1
20	Basal processes of tibial spine AD: 0, absent; 1, present
21	Number of dorsoapical setae on first hind tarsomere: 0, two; 1, absent

Table 5.1 (cont.)

22	Presence of two longitudinal rows of ventral setae on first hind tarsomere: 0, present; 1, absent
23	Pecten of first hind tarsomere with lateral setae longer than median ones : 0, longer; 1, almost same length
24	Shape of hind tibia: 0, flattened; 1, rectangular in cross section
Forewing	
25	Number of apical cells brodering appendix: 0, two ; 1, three
26	Subapical cell: 0, present; 1, absent
27	Presence of third subapical cell: 0, absent; 1, present
28	Forewing with third apical cell: 0, paralle; 1, diverging
29	Forewing with second apical cell: 0, diverging ; 1, parallel; 2 converging
30	Presence of appendix: 0, present; 1, absent
Male genitalia	
31	Anal collar: 0, present wide; 1, present, narrow
32	Ventra extension of anal collar: 0, present; 1, absent
33	Setae on pygofer: 0, absent or very short; 1, present
34	Pygofer process: 0,absent; 1, present
35	Shape of pygofer process: 0, posterodorsal,falcate, elongate; 1, posterodorsal falcate with ventral process; 2, tooth like; 3, basiventral arising dorsally; 4, basiventral arising ventrally, apex curved; 5, posteroventral, extending dorsad
36	Orientation of tooth like process:0, posterad; 1, mesad; 2, dorsad
37	Apex of posterodorsal process:0, elongate, attenuate; 1, elongate, blunt tip; 2, abruptly tapered and hooked; 3, distinctly bent ventromesad; 4, short and broad; 5, forked; 6, curved ventrad with dorsal process
38	Macrosetae subgenital plate : 0, absent; 1, present
39	Position of macrosetae: 0, submarginal; 1, mesal; 2, both submarginal and mesal; 3, median; 4, restricted to base; 5; restricted to apex
40	Submarginal macrosetae: 0, extending morethan half of length of subgenital plate; 1, restricted to apical half

Table 5.1 (cont.)

41	Submarginal row of macrosetae: 0, bi seriate or multiseriate; 1, uniseriate
42	Mesal row extending: 0, extending beyond half; 1, restricted to half
43	Truncate pex of subgenital plate: 0, present; 1, absent
44	Apex of subgenital plate: 0, abruptly curved dorsad; 1, Not curved
45	Fine setae: 0,present long; 1, short
46	Lobe that attaches to style: 0, well developed and produced; 1, poorly developed or absent
47	Subgenital plate:0, dorsoventrally compressed; 1, dorsolaterally compressed
48	Mesal margin of subgenital plate expanded or pointed at the middle: 0, present; 1, absent
49	Style apodeme: 0, narrow 1, broad and (ray- like markings)
50	Style apex: 0, acute; 1, acuminate; 2, hooked; 3, foot like; 4, broad, abruptly curved dorsad with processes; 5, broad with ventral process; 6, hooked ventrad; 7, expanded apically
51	Lateral lobe on inner margin of style: 0, absent 1, present
52	Presence of long setae on style:0, present; 1, absent; 2, present on dorsal hump
53	Spines on style apex: 0, present; 1, absent
54	Presence of short setae on style: 0, present along dorsal or dorsolateral margin; 1, present along ventral surface; 2, absent
55	Texture of ventral margin of apophysis of style: 0, smooth; 1, serrated
56	Presence of spine on ventral margin of style posterad of joint with connective: 0, present; 1, absent
57	Connective 0, V- shaped; 1, U- shaped; 2, U shaped with short median arm; 3, T- shaped with long arm; 4, T-shaped with short arm
58	Anterior margin of U- shaped connective: 0, deeply concave 1, narrowly concave
59	Connection of aedeagus with connective: 0, articulate 1, fused
60	Keel on connective: 0, absent 1, two; 2, one
61	Presence of anterior projection on connective: 0, absent; 1, present
62	Presence of apical processes: 0, present; 1, absent 2, reduced to spine
63	Number of apical processes: 0, paired symmetrical; 1, paired asymmetrical; 2, two pairs ; 3,more than two pairs

Table 5.1 (cont.)

64	Position of gonopore with respect to that of apical processes: 0, above processes or at extreme apex; 1, below processes
65	Aedeagus pustulate: 0, absent; 1, present

Table 5.2. Data matrix of 65 characters of 42 taxa

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Macropsinae	2	1	1	4	0	1	?	0	0	1	?	0	0	0	?	?	2	1	1	0
Chiasmodolon sp.	0	0	0	4	1	0	0	1	1	0	0	0	1	0	3	?	2	0	0	0
Rotundicerus luteus	0	0	0	4	0	0	1	0	0	0	0	1	0	0	3	1	0	0	0	0
Gen. nov 1 (Borneo)	0	0	0	?	0	0	0	0	1	0	1	0	0	0	3	2	1	0	1	0
Gen. nov.2 (Madagascar)	0	0	0	4	0	0	0	0	1	0	1	1	0	0	3	0	0	0	1	0
Ceylonoscopus	0	0	0	4	0	0	0	0	1	0	1	1	0	0	3	1	0	0	1	0
Kopamerra sp.	0	0	0	6	0	0	0	1	0	0	1	0	0	0	2	0	0	0	0	1
Pretioscopus sp.	0	0	0	4	0	0	1	1	0	0	1	0	0	0	2	0	0	0	1	0
Idioscopus grossus	1	0	0	2	1	0	1	0	0	0	0	0	0	0	2	0	0	0	1	0
nr. *Idioscopus inornatus*	1	0	0	5	0	0	1	1	0	0	2	0	0	0	2	1	0	0	1	0
N. convolutus	0	0	0	2	0	0	0	1	0	0	0	0	0	0	2	1	1	0	1	0
N.trimaculatus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	2	0	0	0	1	0
N. flexus	0	0	0	1	0	0	0	1	0	0	1	0	0	1	2	1	0	0	1	0
N. asymmetricus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0
N. compressus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0
N. uniprocessus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. quinquespinosus	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0
N. tetanus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. latus	0	0	0	?	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. basiprocessus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0
N. breviprocessus	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0	1	0	1	0
N. serratus	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0	1	0	0	0
N. littoralis	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0
N. bifurcatus	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. ranfomafanaensis	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0

Table 5.2. (cont.)

N. affinis	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. orbiculatus	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. mananarensis	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. fasika	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. tulearensis	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. nigrum	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. unimaculatus	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0
N. nanus	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0
N. dentatus	0	0	0	3	0	0	0	1	0	0	1	0	0	0	1	2	1	0	1	0
N. brevipinguis	0	0	0	3	0	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0
N. trilineatus	0	0	0	3	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
N. recurvus	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
N. dorsalis	0	0	0	2	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0
N. tristriatus	0	0	0	3	0	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0
N. sclerophyllus	0	0	0	2	0	0	0	1	0	0	1	0	0	0	1	2	1	0	1	0
N. angulatus	0	0	0	3	0	0	0	1	0	0	1	0	0	0	1	2	1	0	1	0

Table 5.2. (cont.).

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Macropsinae	2	0	2	1	?	0	1	1	0	1	2	1	0	1	5	?	?	0	?	?
Chiasmodolon sp.	0	1	0	0	0	0	0	0	0	0	0	1	0	1	5	?	?	1	5	?
Rotundicerus luteus	0	1	1	0	0	0	0	1	0	0	0	1	0	0	?	?	?	0	?	?
Gen. nov 1 (Borneo)	0	0	1	0	1	1	0	1	0	0	0	0	0	2	4	?	?	0	?	?
Gen. nov.2 (Madagascar)	0	1	1	0	1	1	0	1	0	0	1	0	0	0	?	?	?	0	?	?
Ceylonoscopus	0	1	1	0	1	1	0	1	1	0	0	0	0	2	4	?	?	1	4	?
Kopamerra sp.	0	1	1	0	0	0	1	0	0	0	0	1	0	0	?	?	?	0	?	?
Pretioscopus sp.	0	1	1	0	0	0	1	0	0	0	1	0	0	0	?	?	?	0	?	?
Idioscopus grossus	0	1	1	0	0	1	1	0	1	0	1	1	0	0	?	?	?	0	?	?
nr. *Idioscopus inornatus*	0	1	1	0	0	0	0	0	0	0	1	1	0	1	2	0	?	0	?	?
N. convolutus	1	0	0	0	1	0	0	1	2	0	0	1	0	1	2	0	?	1	2	0
N.trimaculatus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	2	?	?	1	2	1
N. flexus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	2	1	?	1	2	0
N. asymmetricus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	2	1	?	1	2	0
N. compressus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	0	?	?	1	3	?
N. uniprocessus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	2	0	?	1	2	0
N. quinquespinosus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	3	?	?	1	2	0
N. tetanus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	?	?	6	1	2	0
N. latus	1	0	0	0	1	1	0	1	1	0	0	1	0	1	2	0	?	1	2	0
N. basiprocessus	1	0	0	0	1	1	0	1	1	0	0	1	0	1	2	0	?	1	2	0
N. breviprocessus	1	0	0	0	1	1	0	1	2	0	0	1	0	1	2	0	?	1	2	0
N. serratus	1	0	0	0	1	1	0	1	?	0	0	1	0	1	2	0	?	1	1	?
N. littoralis	1	0	0	0	1	0	0	1	2	0	0	1	0	1	2	1	?	1	2	0
N. bifurcatus	1	0	0	0	1	0	0	1	2	0	0	1	1	1	0	?	5	1	2	0
N. ranfomafanaensis	1	0	0	0	1	0	0	1	2	0	0	1	1	1	0	?	1	1	2	0
N. affinis	1	0	0	0	1	0	0	1	2	0	0	1	1	1	0	?	1	1	2	0

Table 5.2 (cont.).

N. orbiculatus	1	0	0	0	1	0	0	1	2	0	0	1	1	1	0	?	3	1	2	0
N. mananarensis	1	0	0	0	1	0	0	1	2	0	0	1	0	1	0	1	3	1	2	0
N. fasika	1	0	0	0	1	0	0	1	2	0	0	1	1	1	0	?	4	1	2	0
N. tulearensis	1	0	0	0	1	0	0	1	2	0	0	1	1	1	1	?	?	1	2	0
N. nigrum	1	0	0	0	1	?	0	1	2	0	0	1	1	1	1	?	?	1	2	0
N. unimaculatus	1	0	0	0	1	0	0	1	2	0	0	1	1	1	0	?	5	1	2	0
N. nanus	1	0	0	0	1	1	0	1	2	0	0	1	1	1	0	?	5	1	2	0
N. dentatus	1	0	0	0	1	1	0	1	2	0	0	1	1	1	0	?	1	1	2	0
N. brevipinguis	1	0	0	0	1	1	0	1	1	0	0	1	1	1	1	?	?	1	0	1
N. trilineatus	1	0	0	0	1	1	0	1	2	0	0	1	1	1	0	?	1	1	0	1
N. recurvus	1	0	0	0	1	1	0	1	2	0	0	1	1	1	0	?	2	1	0	1
N. dorsalis	1	0	0	0	1	1	0	1	2	0	0	1	1	1	0	?	2	1	0	1
N. tristriatus	1	0	0	0	1	1	0	1	2	0	0	1	1	1	0	?	0	1	0	1
N. sclerophyllus	1	0	0	0	1	1	0	1	2	0	0	1	1	1	0	?	0	1	0	0
N. angulatus	1	0	0	0	1	0	0	1	2	0	0	1	1	1	0	?	0	1	0	0

Table 5.2 (cont.).

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Macropsinae	?	?	?	?	0	1	1	?	0	2	1	3	1	0	0	1	3	?	0	0
Chiasmodolon sp.	?	?	1	1	0	0	0	?	1	6	3	2	1	1	0	1	2	?	0	2
Rotundicerus luteus	?	?	?	?	0	1	2	?	0	6	3	2	1	1	0	1	2	?	0	2
Gen. nov 1 (Borneo)	?	?	?	?	0	1	1	?	0	1	1	1	1	1	0	0	4	?	0	2
Gen. nov.2 (Madagascar)	?	?	?	?	0	1	0	?	0	1	1	1	1	1	0	0	3	?	0	2
Ceylonoscopus	?	?	1	1	1	1	0	?	0	1	1	1	1	1	0	0	3	?	0	2
Kopamerra sp.	?	?	?	?	0	1	1	?	0	3	1	1	1	0	0	1	4	?	0	2
Pretioscopus sp.	?	?	?	?	0	1	1	?	0	1	2	1	1	0	1	1	4	?	0	2
Idioscopus grossus	?	?	?	?	0	1	1	?	0	7	1	0	1	0	0	1	4	?	0	2
nr. *Idioscopus inornatus*	?	?	?	?	0	1	1	?	0	8	1	0	1	0	1	1	4	?	0	2
N. convolutus	1	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N.trimaculatus	1	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. flexus	0	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. asymmetricus	1	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. compressus	?	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. uniprocessus	1	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. quinquespinosus	1	1	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. tetanus	1	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. latus	1	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. basiprocessus	1	0	1	1	1	0	0	1	0	2	0	1	1	2	0	1	0	?	0	0
N. breviprocessus	1	0	1	1	1	0	0	1	?	2	0	1	1	2	0	1	0	?	0	0
N. serratus	1	0	1	1	1	0	0	1	1	2	0	1	1	2	0	1	0	?	0	0
N. littoralis	0	0	1	1	1	1	0	1	0	?	0	1	1	2	0	1	1	0	1	1
N. bifurcatus	0	1	1	1	1	1	0	1	1	0	0	1	1	2	0	1	1	0	1	1
N. ranfomafanaensis	0	1	1	1	1	1	0	1	1	1	0	1	1	2	0	1	1	0	1	1
N. affinis	0	1	1	1	1	1	0	1	1	1	0	1	1	2	0	1	1	0	1	1

Table 5.2 (cont.).

N. orbiculatus	0	1	1	0	1	1	0	0	1	?	0	1	1	2	0	1	1	0	1	1
N. mananarensis	0	1	1	1	1	1	0	0	1	1	0	1	1	2	0	1	1	0	1	1
N. fasika	1	1	1	1	1	1	0	1	1	4	0	1	1	2	0	1	1	1	1	1
N. tulearensis	0	1	0	1	1	1	0	1	1	4	0	1	1	2	0	1	1	1	1	1
N. nigrum	0	1	0	1	1	1	0	1	1	4	0	1	1	2	0	1	1	1	1	1
N. unimaculatus	1	0	1	1	0	1	0	1	1	1	0	1	1	2	0	1	1	0	1	1
N. nanus	0	0	1	1	0	1	0	1	1	0	0	1	1	2	0	1	1	0	1	1
N. dentatus	0	1	1	1	0	1	0	1	1	1	0	1	0	2	0	1	1	0	1	1
N. brevipinguis	0	?	1	1	0	1	0	1	1	?	0	1	1	2	0	1	1	0	1	1
N. trilineatus	1	?	1	1	0	1	0	1	1	1	0	1	1	2	0	1	1	0	1	1
N. recurvus	1	?	1	1	0	1	0	1	1	5	0	1	1	2	0	1	1	0	1	1
N. dorsalis	1	?	1	1	0	1	0	1	1	?	0	1	1	2	0	1	1	0	1	1
N. tristriatus	1	?	1	1	0	1	0	1	1	1	0	1	1	2	0	1	1	0	1	1
N. sclerophyllus	1	?	1	1	0	1	0	1	1	1	0	1	1	2	0	1	1	0	1	1
N. angulatus	1	?	1	1	0	1	0	1	1	1	0	1	1	2	0	1	1	0	1	1

Table 5.2 (cont.).

	61	62	63	64	65
Macropsinae	0	1	?	?	0
Chiasmodolon sp.	0	1	?	0	0
Rotundicerus luteus	0	1	?	0	1
Gen. nov 1 (Borneo)	1	1	?	0	0
Gen. nov.2 (Madagascar)	1	1	?	?	0
Ceylonoscopus	1	0	0	0	0
Kopamerra sp.	0	0	2	2	0
Pretioscopus sp.	1	1	?	?	0
Idioscopus grossus	0	0	0	2	0
nr. *Idioscopus inornatus*	0	0	0	1	0
N. convolutus	0	2	0	0	0
N.trimaculatus	0	?	?	0	0
N. flexus	0	0	1	0	0
N. asymmetricus	0	0	1	2	0
N. compressus	0	?	?	0	0
N. uniprocessus	0	?	?	0	0
N. quinquespinosus	0	0	3	2	0
N. tetanus	0	0	?	2	0
N. latus	0	?	0	0	0
N. basiprocessus	0	?	?	0	0
N. breviprocessus	0	0	0	0	0
N. serratus	0	0	?	0	0
N. littoralis	0	0	0	1	0
N. bifurcatus	0	0	2	1	0
N. ranfomafanaensis	0	0	0	1	0
N. affinis	0	0	0	1	0

Table 5.2 (cont.).

N. orbiculatus	0	0	0	1	0
N. mananarensis	0	0	0	1	0
N. fasika	0	0	0	1	0
N. tulearensis	0	0	0	1	0
N. nigrum	0	0	0	1	0
N. unimaculatus	0	0	?	1	0
N. nanus	0	0	0	1	0
N. dentatus	0	0	0	1	0
N. brevipinguis	0	0	?	0	0
N. trilineatus	0	0	0	1	0
N. recurvus	0	0	0	1	0
N. dorsalis	0	0	0	1	0
N. tristriatus	0	?	0	1	0
N. sclerophyllus	0	0	0	1	0
N. angulatus	0	0	0	1	0

Table 5.3. List of apomorphies for nodes of cladogram in figure 5.17. Characters are listed with states in parentheses.

Nodes	Subtending apomorphies
1	4(2): 8(1): 35(2): 57(0): 62(0)
2	9(1): 15(3): 16(1): 22(1): 39(4): 54(1): 60(2)
3	11(0): 19(0): 39(5): 50(6): 51(3): 52(2): 57(2)
4	25(1): 26(1): 32(0): 34(2): 35(4): 56(0): 61(1)
5	12(1)
6	22(1): 27(1): 28(0): 34(0): 47(1): 57(4): 60(2): 64(2)
7	7(1): 31(1): 55(1)
8	1(1): 11(0): 50(7): 52(0)
9	21(1): 23(0): 25(1): 29(2): 38(1): 45(1): 51(0): 54(2)
10	37(6): 41(1): 46(0): 50(2)
11	4(1): 26(1)
12	15(1)
13	16(1): 36(1): 63(1)
16	63(3)
17	35(3): 64(2)
18	29(1)
19	17(1)
20	14(1): 29(2): 49(1)
21	15(1): 36(1): 57(1): 60(1): 64(1)
22	33(1): 35(0): 49(1):
23	42(1)
24	37(1)
26	37(3)
27	48(0)
28	37(4): 50(4): 58(1)
29	35(1): 43(0)
30	4(0): 45(0)

Table 5. 3 (cont.)

31	17(1): 26(1):
32	4(3): 37(1): 42(1)
33	39(0): 40(1)
34	41(1)
34	41(1)
35	17(0)
36	4(2): 15(0): 37(2): 50(5)
37	37(0)
38	16(2): 40(0)
Macropsinae	1(2); 2(1); 3(1); 6(1); 10(1); 17(2); 18(1) ;21(2) ;23(2) ;24(1); 27(1); 30(1); 31(2); 47(1); 50(2); 52(3)
Chiasmodolon sp.	5(1): 8(1): 13(1): 17(2): 23(0): 28(0): 38(1): 46(0): 49(1)
Rotundicerus luteus	7(1): 9(0): 12(1): 34(0): 47(2): 65(1)
Gen. nov. 1	16(2); 17(1); 22(0); 47(1); 57(4)
Gen. nov. 2	16(0); 31(1); 34(0)
Ceylonoscopus	29(1); 38(1); 45(1); 62(0)
Kopamerra sp.	4(6): 19(0): 20(1): 50(3): 63(2)
Pretioscopus sp.	4(4): 32(0): 51(2): 61(1): 62(1)
Idioscopus grossus	5(1): 8(0): 26(1): 29(1): 55(0)
nr. *Idioscopus inornatus*	4(5): 11(2): 16(1): 27(0): 34(1): 50(8): 64(1)
N. convolutus	11(0): 16(1): 17(1): 62(2)
N. trimaculatus	40(1)
N. flexus	14(1): 15(2): 41(0)
N. asymmetricus	64(2)

Table 5. 3 (cont.)

N. compressus	35(0): 39(3)
N. quinquespinosus	11(0): 42(1)
N. serratus	19(0): 39(1)
N. littoralis	4(1): 11(0)
N. bifurcatus	50(0): 63(2)
N. orbiculatus	44(0)
N. mananarensis	33(0)
N. fasika	41(1)
N. unimaculatus	15(0): 41(1)
N. nanus	50(0)
N. dentatus	16(2): 53(0)
N. brevipinguis	29(1): 35(1): 64(0)
N. recurvus	11(0)
N. angulatus	26(0)
N. sclerophyllus	4(2)
Autoapomorphies of *N. uniprocessus, N. tetanus, N. latus, N. basiprocessus, N. breviprocessus, N. ranomafanaensis, N. affinis, N. tulearensis, N. nigrum, N. trilineatus, N. dorsalis, N. tristriatus* are not included.	

Chapter 6

Review of Mileewine Leafhoppers (Hemiptera: Cicadellidae: Mileewinae) in Madagascar With Description of Seven New Species[2]

Abstract

The leafhopper subfamily Mileewinae was previously known in Madagascar from a single species, *Ujna flavidipes* Distant, also recorded from Seychelles. Study of samples from a recently completed Arthropod biodiversity inventory in Madagascar revealed seven new, apparently endemic species distinguishable based on external and genitalia characters and all referable to the genus *Ujna* Distant based on the present definition of the genus: ***U. variabilis*** sp. nov., ***U. bimaculata*** sp. nov., ***U. affinis*** sp. nov., ***U. trishula*** sp. nov., ***U. acuta*** sp. nov., ***U. alba*** sp. nov., ***U. rostrata*** sp. nov. are described and illustrated. *U. flavidipes* Distant is redescribed and illustrated. Three informal species groups are proposed. A key to the males of *Ujna* from Madagascar is presented. A key to world genera of Mileewinae and a morphological comparison of *Ujna* with other genera are also provided. Two new placements of genera are proposed: *Processina* Yang, Deitz and Li is transferred from tribe Cicadellini to Mileewini (Mileewinae) **placement n.** and *Archeguina* Young is transferred from Mileewini to Cicadellini (Cicadellinae) **placement n.**

Introduction

The leafhopper subfamily Mileewinae is recorded from the Ethiopian, Oriental, and Neotropical regions. Species of this group inhabit wet tropical forests and appear to be most diverse and abundant in montane cloud forests. Evans (1947) first recognized the group as a distinct taxon when he erected the tribe Mileewanini [sic!] including two valid genera *Mileewa* Distant and *Ujna* Distant and placed it in the subfamily Cicadellinae (as "Tettigellinae") based on the dorsal ocelli, narrow gena, and convex frontoclypeus. He distinguished the tribe from other Cicadellinae by the reduced forewing clavus, wide appendix, and lack of crossvein r-m1 (as "M1+2"). Later, Young (1965, 1993) added two more genera, *Amahuaka* Melichar and *Archeguina* Young from the Neotropics and New Guinea, respectively. Chiang and Knight

[2]This chapter appeared in its entirety in the journal of *Annals of Entomological Society of America* as Krishnankutty, S. M and Dietrich, C. H. 2011. Review of leafhopper tribe Mileewini (Hemiptera: Cicadellidae) from Madagascar with description of seven new species. 104 (4): 636- 648. . This article is reprinted withthe permission of the publisher and is available from http://www.ingentaconnect.com/ and DOI: http://dx.doi.org/10.1603/AN11022

(1991) in their revision of Mileewini (as "Mileewanini") of Taiwan synonymized *Formotettigella* Ishihara under *Mileewa*. Young (1993) emended the spelling of Mileewanini to Mileewini in accordance with Article 32(c) (iii) of the International Code of Zoological Nomenclature. Gebicki and Szwedo (2001) described two additional genera, each represented by a single species from Eocene Baltic amber.

Previous authors have disagreed over the subfamily placement of Mileewini. Young (1965) transferred the tribe from Cicadellinae to subfamily Typhlocybinae. Later, Mahmood (1967) expressed doubt about Young's placement of this tribe but did not explicitly exclude it from Typhlocybinae. Mahmood and Ahmed (1968) in their work on higher classification problems in Typhlocybinae moved the tribe back to subfamily Cicadellinae. Linnavuori and DeLong (1977) were the first to treat Mileewinae (as "Mileewaninae") as a separate subfamily and this status was accepted by Dietrich (2005). Recent phylogenetic analyses based on morphological and molecular data, recovered Mileewinae as sister to Typhlocybinae (Balme 2007; Dietrich et al. 2010). We continue to recognize subfamily Mileewinae, containing only the nominotypical tribe Mileewini.

Mileewinae may be distinguished based on the following combination of characters: head with frontoclypeus strongly convex, rostrum extended to or beyond mesothoracic trochanters, ocelli on crown well separated from eyes; forewing vein R two-branched or unbranched, without closed subapical cells, appendix well developed (Fig. 6.1); hind wing with submarginal vein on or very near apical margin; profemur with >6 fine setae in intercalary row and 2 ventroapical macrosetae (AM1 and AV1); hind femoral setal formula 2:1:1; male subgenital plate slender, parallel sided or gradually tapered through most of length, with longitudinal row or band of macrosetae distant from lateral margin. This definition excludes *Archeguina* Young, which is more appropriately placed in Cicadellini (new placement), but encompasses *Processina* Yang, Deitz and Li (new placement) which was previously unplaced to a tribe within Cicadellinae (Yang et al. 2005).

The genus *Ujna* Distant (type species: *Ujna delicatula* Distant) comprises seven previously described species, distributed in the Oriental region and Madagascar (Rao 1995). In Madagascar, this genus was represented by a single species, *U. flavidipes* Distant, originally described from Seychelles (Evans 1953). The present study revealed seven new species of this genus from Madagascar based on external and genitalia characters.

Keys to the genera of Mileewinae and to males of the species of *Ujna* from Madagascar are presented. *Ujna flavidipes* Distant is redescribed and illustrated.

Materials and Methods

Specimens studied are primarily from material collected during the Terrestrial Arthropod Inventory of Madagascar project conducted by the California Academy of Sciences (CAS) and Tsimbazaza Botanical and Zoological Park (PBZT). The holotype of *U. flavidipes* deposited in The Natural History Museum, London and additional pinned specimens of *U. flavidipes* (identified by Evans) from the Muséum national d'Histoire naturelle, Paris were also examined. Morphological terminology follows Dietrich (2005) and Anufriev and Emeljanov (1988) for external and male genitalia characters and Davis (1975) for female genitalia. Habitus photos were taken using a Microptics imaging system (Photografix, Richmond, Virgina). Illustrations of genitalia were made using a camera lucida attached to a compound microscope and line drawings were traced using Adobe Illustrator ver. 12.0.1.

Holotypes and paratypes are deposited in the California Academy of Sciences, San Francisco (CAS) and Illinois Natural History Survey, Champaign (INHS) respectively, unless otherwise indicated. The label information is provided as 'materials examined'. The numbers prefixed with "MA" (Madagascar) and "BLF" (Brian L. Fisher) are collection event codes and those with "CASENT" (California Academy of Sciences Entomology) are unique identification numbers for the individual specimens.

RESULTS

Key to the genera of Mileewinae

1. Male pygofer with dorsoapical process; New World distribution…….***Amahuaka*** **Melichar**

— Male pygofer without process or with ventral process extending posterodorsally; Old World distribution…2.

2. Dorsal coloration mostly dull stramineous; rostrum extended beyond hind trochanters; aedeagal shaft with posteroventral thornlike process; style apex in dorsal view rounded, with apical setae ….. ***Processina*** **Yang, Deitz & Li**

— Dorsal coloration dark brown marked with white (rarely mostly white); rostrum not surpassing mesothoracic trochanters; aedeagal shaft without thornlike posteroventral process; style apex in dorsal view pointed, without apical setae …. 3

3. Forewing apex truncate or emarginate; male style with long setae near middle; female first valvulae with columns of dorsal sculpture vertical or curved anteroventrad; second valvulae broad throughout length with abruptly pointed apex; head distinctly narrower than pronotum (ratio ~ 0.8:1) ….***Mileewa*** **Distant**

— Forewing apex rounded (Fig. 6.1); male style without long setae; female first valvulae with columns of dorsal sculpture oriented posteroventrad (Fig. 6.5B) or without uniform orientation; second valvulae gradually narrowed towards apex (Fig. 6.6A); head width subequal to that of pronotum (ratio 1:1 or 0.9:1) … ***Ujna*** **Distant**

Ujna **Distant**

Type species. *U. delicatula* Distant, 1907: 239

Diagnosis. *Ujna* may be distinguished from other mileewine genera based on the following combination of characters: Dorsal coloration dark brown with white markings (rarely mostly white); head width subequal to that of pronotum; forewing apex round (Fig.6.1); male pygofer with process arising ventrally (Fig. 6.3A) or absent; subgenital plate slender, gradually narrowed to apex; style without long setae near middle, with or without preapical tooth; female first valvulae with dorsal sculpturing imbricate, with columns of cells inclined posteroventrad (Fig. 6.5B); second valvulae variable interspecifically in shape, apex gradually tapered (Fig. 6.6B); female seventh sternum with caudal margin usually produced medially.

Distribution. Myanmar (Burma), Tamil Nadu (India), Madagascar, Luzon (Philippines), Mahe Islands (Seychelles), Sri Lanka and Thailand.

Notes. *Ujna* and *Mileewa* are very closely related genera with overlapping distribution in the Oriental region. The distinction between these two genera has been interpreted differently by various authors. According to Distant (1908), species of *Mileewa* have the vertex with median carination and the forewing truncate, while *Ujna* has the face with distinct median carination and the forewing rounded. Our studies of these two genera, including Distant's type material, indicate that neither genus has distinct carinae on the crown or face. Baker (1914) noted that the head characters vary interspecifically and distinguished *Mileewa* from *Ujna* based on the shape

of the forewing apex. The diagnosis of *Ujna* presented above is based on examination of specimens from the type series of *U. delicatula* from Sri Lanka, *Ujna* spp. from Thailand and India, and the eight species presently recorded from Madagascar.

The male genitalia of *Ujna* spp. closely resemble those of *Mileewa* spp but differ in the absence of long setae on the style. The female genitalia of these two genera also differ, as indicated in the key, and three groups of *Ujna* species from Madagascar are diagnosable based on ovipositor structure, as described below. The female genitalia of *Ujna flavidipes* closely resemble those of the Neotropical genus *Amahuaka* in overall shape and sculpturing of the first and second valvulae and in the presence of a dorsal notch on the latter, but males may be distinguished based on the different positions of the pygofer process and by the shape of the subgenital plate. Although, the above-mentioned characters may be used to separate these related genera, the extensive variation found in the female genitalia, which have not previously been studied in this subfamily, accentuates the need for a comprehensive phylogenetic study to better elucidate the status of the currently recognized genera.

The eight mileewine species described from Madagascar are tentatively placed in the genus *Ujna* based on their overall similarity to the type species, *U. delicatula*. As in *U. delicatula*, all Malagasy species have a round forewing apex and pronotal width subequal to head width. The male genitalia are similar in having the pygofer with a ventral process (except the *rostrata* group) and the style with a preapical tooth (except the *acuta* and *rostrata* groups). In the female genitalia, the first and second valvulae of *U. flavidipes* and *U. affinis* closely resemble those of *U. delicatula*. However, none of the Malagasy species has the general color pattern of the type species. Also, the female genitalia of the *acuta* and *rostrata* groups differ considerably from those of the type species. Given such variation, it may eventually be appropriate to establish additional genera for some species of Mileewinae from Seychelles and Madagascar. However, this should be done within the context of a comprehensive treatment of the world fauna of the group.

The species of *Ujna* from Madagascar may be divided into three species groups based on the external and genitalia characters. The proposed species groups are *flavidipes* group, *rostrata* group and *acuta* group (see diagnostic characters with included species descriptions below).

Key to males of the species of *Ujna* from Madagascar

1. Pronotal median length equal to or shorter than that of crown; forewing black with hyaline circular spots or streaks; male abdominal third sternum (3S) apodeme reduced or absent; male pygofer posterior margin truncate, with ventral process (Figs. 6.3A; 6.4A)…….**2**

— Pronotal median length longer than that of crown; forewing white or pale brown without hyaline spots; male abdominal 3S apodeme well developed, elongate, extending beyond sixth segment; male pygofer posterior margin not truncate, without ventral process (Figs. 6.4M, S) (*rostrata* group) …**7**

2. Ocelli located approximately on line between anterior eye angles (Figs. 6.2A, C); subgenital plate with two apical setae longer than series of setae on ventral surface (Fig. 6.3B); male pygofer ventral process short, hooked ventrad (Fig. 6.3A); style with preapical tooth, forming foot shaped apex; aedeagus in lateral view with both ventral and dorsal processes (Fig. 6.3E) (*flavidipes* group) ……..**3**

— Ocelli located well anterad of line between anterior eye angles (Figs. 6.2M, O); subgenital plate without long apical setae (Fig. 6.4B); male pygofer with ventral process acuminate, extended posterodorsad (Fig. 6.4A); style apex without preapical tooth, apex beak shaped (Fig. 6.4C); aedeagus in lateral view without processes (Fig. 6.4E) or with dorsal process only (Fig. 6.4K) (*acuta* group) … **6**

3. Forewing with three apical cells (Ra vein absent) …. **4**

— Forewing with four apical cells (Ra vein present) (Fig. 6.1) ……**5**

4. Male pygofer without macrosetae (Fig. 6.3L); aedeagus with short dorsal process arising near middle of shaft (Fig. 6.3O)… ***U. variabilis*** sp. nov.

— Male pygofer with macrosetae (Fig. 6.3Q); aedeagus with long dorsal process arising near apex of shaft (Fig. 6.3U)….***U. bimaculata*** sp. nov.

5. Male pygofer with macrosetae; aedeagus with dorsal cleft near the base of shaft (Fig. 6.3E); face without basal black marking (Fig. 6.2B) … ***U. flavidipes*** Distant

— Male pygofer without macrosetae; aedeagal shaft smooth without dorsal cleft (Fig.6. 3J); face with basal black marking extending from crown (Fig. 6.2D)….***U. affinis*** sp. nov.

6. Aedeagus without processes (Fig. 6.4E); male pygofer with long process extending beyond posterior margin (Fig. 6.4A) .. ***U. trishula*** sp. nov.

— Aedeagus with dorsal processes (Fig. 6.4K); male pygofer with short process not extending beyond posterior margin (Fig. 6.4G) .. ***U. acuta*** sp. nov.

7. Body mostly pale white with black marking confined to forewing apex and clavus (Fig. 6.2S); male pygofer with posterior margin rounded (Fig. 6.4S); aedeagus in lateral view with base broader than apex, with process arising near dorsal margin (Fig. 6.4V) … ***U. alba*** sp. nov.

— Body mostly light brown with white markings on crown (Fig. 6.2Q); male pygofer with posterior margin slightly pointed (Fig. 6.4M); aedeagus in lateral view with base as broad as apex, process arising below dorsal margin (Fig. 6.4Q)… ***U. rostrata*** sp. nov.

SPECIES GROUPS

1) *flavidipes* group

(*U. flavidipes* Distant, *U. affinis* sp. nov., *U. variabilis* sp. nov. and *U. bimaculata* sp. nov.)

Diagnosis. Ocelli located on or close to line between anterior eye angles, anterior margin of crown broadly pointed, interocular width almost half crown median length. Male (Figs. 6.3A-V) pygofer in lateral view with truncate posterior margin and short, falcate ventral process, in dorsal view with broad sclerotization extending to the anterior margin of membranously attached anal segment; subgenital plate with two apical setae in addition to preapical series of macrosetae on ventral surface; style apex footlike with preapical tooth, male abdominal apodeme reduced. Female first valvulae (Figs. 6.5A-F) with dorsal sculpturing imbricate, ventral area with strigate sculpturing in distal two thirds. Second valvulae (Figs. 6.6A-F) with distinct dorsal notch near midlength, ducts conspicuous, dorsal teeth small and closely spaced, confined to apical half and extending to ventral margin near apex. Seventh sternum with caudal margin produced medially.

***Ujna flavidipes* Distant**

Figs. 6.2A, B; Figs. 6.3A–E; Figs. 6.5A, B; **Figs.6. 6A, B**

Diagnosis. This species may be separated from other species in the *flavidipes* group by the following combination of characters: male pygofer with macrosetae, style with preapical tooth farther from apex, aedeagus with dorsal margin depressed near socle.

Length of male, 4.1 mm; female, 4.6–4.8 mm.

Coloration. Crown black with white spots; one median, one pair close to ocellus, and another pair near hind margin of crown. Pronotum, mesonotum, and scutellum black. Forewing black with hyaline markings; one near costal margin, one at apex of clavus and one at base of second and third apical cell. Face, venter of thorax and legs white without markings.

Structure. Crown broadly produced in dorsal aspect, ocelli located on line between anterior eye angles. Laterofrontal suture extending onto crown and attaining ocelli. Face with muscle impressions distinct. Pronotum with indistinct striations on posterior half, median length shorter than that of crown. Forewing with appendix broader near apex; veins distinct; with four apical cells, base of Ra (Fig. 6.1) located proximad of base of third apical cell; length of second apical cell half that of first apical cell; costal area opaque proximad of apical cells.

Male Genitalia. Pygofer with macrosetae, posterior margin truncate, with dorsally directed hooked ventral process. Subgenital plate gradually narrowed to apex, with series of macrosetae on ventral surface from base to apex and pair of long apical setae. Style elongate, with preapical tooth. Aedeagus in lateral view elongate, with depression on dorsal margin near socle, with pair of preapical ventral spines and pair of apical dorsal processes.

Female Genitalia. First valvulae in lateral aspect broadly expanded in apical half and tapered preapically to attenuate apex, with imbricate sculpturing throughout dorsal area and extending to ventral margin near apex, ventral area with strigate sculpturing in distal two thirds. Second valvulae in lateral aspect short, broad, strongly broadened near midlength and tapered distally, dorsal margin with small prominence basad of broad distal blade forming distinct cleft. Ducts conspicuous, dorsal teeth crenulate, small and closely spaced, confined to apical half and extending to ventral margin near apex.

Materials Examined. 4 males, 3 females, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59”S, 47°25’26”E, 14-23. IV. 2002, R. Harin’Hala, malaise, secondary forest, MA-02-09C-25 (CAS, INHS); 1 male, same label data as previous except 24. VII - 4. VIII. 2002, MA-02-09C-36 (CAS); 1 male, same label data as previous except 12-19. II. 2002, MA-02-09C-16 (INHS); 2 males, 3 females, same data as previous except Vohiparara at broken bridge, 1110 m, 21°13’34”S, 47°22’11”E, 28. XI – 6. XII. 2001, R. Harin’Hala, malaise trap in high altitude rainforest, MA-02-09A-05 (CAS, INHS); 2 females, same data as previous except radio tower at forest edge, 1130 m, 22°15’3”S, 47°24’25”E, 21- 24.II. 2001, R. Harin’Hala, malaise, mixed tropical forest, MA-02-09B-08 (CAS, INHS); 1 female, same label data as previous except 12-19.II. 2002, MA-02-09B-16 (CAS).

***Ujna affinis* sp. nov.**

Figs. 6.2C, D; Figs. 6.3F–K

Diagnosis. This species may be separated from the other species in the *flavidipes* group by the following combination of characters: male pygofer without macrosetae, style with preapical tooth closer to apex, aedeagus with dorsal margin not depressed near socle.

Length of male, 4.1 mm; female, 4.5–4.7 mm.

Coloration. Crown black with median white line exending beyond ocelli, with two pairs of white spots, one close to ocelli, and one along hind margin of crown. Pronotum black, mesonotum black with median white line not extending beyond scutellar suture. Forewing markings as in *U. flavidipes* except marking near apex of clavus bigger. Face mostly pale white with basal black marking extending from crown. Legs white without markings.

Structure. As in *U. flavidipes.*

Male Genitalia. Closely resembling those of *U. flavidipes* but pygofer without macrosetae, style with preapical tooth closer to apex forming bifurcate apex, aedeagus elongate with dorsal margin not depressed near socle.

Female Genitalia. As in *U. flavidipes*

Materials Examined. *Holotype* male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 21°15’59”S, 47°25’26”E, 22-28. XI. 2001, R. Harin’Hala, malaise, secondary forest, MA-02-09C-04 (CAS). *Paratypes*: 2 males,1 female, same data as holotype except 14-23. IV. 2002, MA-02-09C-25 (CAS); 4 males, 3 females, same data as previous except radio tower at forest edge, 1130 m, 22°15’3”S, 47°24’25”E, 28. I. - 4. II. 2002, R. Harin’Hala, malaise, mixed tropical forest, MA-02-09B-14 (CAS, INHS).

Etymology. The specific epithet is derived from the Latin *affinis* meaning similar, referring to similarity with *U. flavidipes* Distant.

***Ujna variabilis* sp. nov.**

Figs.6. 2E–J; Figs. 6.3L–P; Figs. 6.5C, D; Figs. 6.6C, D

Diagnosis. This species is variable in external coloration and markings, but may be separated by the following combination of characters: forewing without Ra vein, aedeagus with broadly rounded apex and short preapical dorsal processes.

Length of male, 3.9–4.0 mm; female, 4.4–4.5 mm.

Coloration. Three distinct color morphs occur. Morph 1: crown black with median white line often extending to mesonotum. Face mostly white with black markings extending from crown. Forewing pale black with one hyaline spot on costal margin near apex. Morph 2: head and face markings as in Morph 1 but forewing with multiple hyaline streaks. Morph 3: mostly creamy white except dark brown marking on posterior margin of crown, pronotum, mesonotum and apex of forewing.

Structure. As in *U. flavidipes* except forewing with three apical cells (Ra absent) and length of second apical cell almost equal to or shorter than that of first apical cell.

Male Genitalia. Pygofer and subgenital plate as in *U. affinis*. Style elongate with preapical tooth inconspicuous forming foot-shaped apex. Aedeagus in lateral view elongate, with slightly expanded round apex, with single ventral preapical spine, pair of dorsal processes arising near middle of dorsal margin of shaft.

Female Genitalia. First valvulae in lateral aspect as in *U. flavidipes* except narrow and elongate throughout length, imbricate sculpture smaller in size. Second valvulae in lateral aspect narrow, elongate, dorsal margin with a cleft at the middle. Ducts and teeth as in *U. flavidipes.*

Materials Examined. *Holotype* male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 21 – 28. I. 2002, 21°15’3”S, 47°24’25”E, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-13 (CAS). *Paratypes*: 1 female, same data as holotype (CAS); 1 male, same data as holotype except 21 – 24. XII. 2001, MA-02-09B-08; 1 female, same data as holotype except 21 – 28. I. 2002, MA-02-09B-13 (INHS); 1 female, same data as holotype except 14 – 21. I. 2002, MA-02-09B -12 (INHS); 1 female, same data as holotype except 12 – 19. II. 2002, MA-02-09B-20 (CAS); 1 male, same data as holotype except Vohiparara, at broken bridge, 1110 m, 31. III. – 8. IV. 2002, 21°13’34”S, 47°22’11”E, M. Irwin, R. Harin'Hala, malaise trap, high altitude rainforest, MA-02-09A-23 (INHS); 1 male, same data as holotype except Bevaohazo-Ranomana, 970 m, XI – 2000, 21°12’07”S, 47°28’56”E, R. Harin’Hala & M.E. Irwin, malaise trap, MA-01-10-01, CASENT 5500760 (CAS); 1 male, MADAGASCAR: Fianarantsoa Parc National Befotaka-Midongy, Papango 27.7 km S Midongy-Sud Mount Papango, 970 m, 13-19. XI. 2006, 23˚50’07”S, 46˚57’49”E, B. L. Fisher et al., malaise, rainforest, BLF14774 (CAS); 1 male, MADAGASCAR: Province Fianarantsoa, Forêt d’Ambalagoavy Nord, Ambahaka, 750 m, X – XI – 2000, 21°44’20”S, 47°24’50”E, R. Harin’Hala & M.E. Irwin, malaise trap, MA-01-11-01,

CASENT3000727 (CAS); 1 male, MADAGASCAR: Province d'Antsiranana, Montaigne Francais, 150 m, 30. I. – 15. II. 2001, 12°19'30"S, 49°20'E, M. Irwin, R. Harin'Hala, malaise, forested limestone ridge, MA-01-06-05 (INHS); 1 female, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 19 – 26. II. 2002, 21°15'59"S, 47°25'12"E, M. Irwin, R. Harin'Hala, malaise, secondary tropical forest, MA-02-09C-17 (INHS); 1 male, same data as previous except Talatakely, 900 m, 9-19. I. 2001, 21°15'S, 47°25'12"E, D. H. & K. M. Kavanaugh, R. L. Brett, E. Elsom, F. Vangas, malaise trap, CASENT 3003364 (CAS).

Etymology. The specific epithet is derived from the Latin *vario* meaning various, referring to different color forms.

***Ujna bimaculata* sp. nov.**

Figs. 6.2K, L; Figs. 6.3Q–V; Figs. 6.5E, F; Figs. 6.6E, F

Diagnosis. This species may be separated by the following combination of characters: forewing with two hyaline elongate spots along costal margin, without Ra vein, aedeagus with broadly rounded apex and apical long curved dorsal processes.

Length of male, 4.7 mm; female, 5.2 mm.

Coloration. Crown black with median circular white spot, two pairs of white markings, one close to ocelli, other near posterior margin of crown. Face and legs white. Forewing with two hyaline elongate spots along costal margin and one at apex of clavus.

Structure. As in *U. flavidipes* except forewing vein Ra absent.

Male Genitalia. Pygofer, subgenital plate, style as in *U. flavidipes*. Aedeagus elongate, in lateral view with broad round apex, with pair of long ventral preapical spines, with pair of long dorsal apical processes.

Female Genitalia. First valvulae in lateral aspect narrow, elongate, with imbricate sculpturing throughout dorsal area and slightly extending to ventral margin near apex. Second valvulae as in *U. variabilis.*

Materials Examined. *Holotype* male, MADAGASCAR: Province d'Antsiranana, Parc National Montagne d'Ambre, 12.2 km 211° SouthWest of Joffreville, 1300 m, 2-7 .II. 2001, 12°35'47"S 49°9'34"E, Fisher, Griswold et al., malaise trap, in ontane rainforest, BLF2853 (CAS). *Paratypes*: 1 male, same data as holotype (INHS), 1 female, MADAGASCAR: Province

d'Antananarivo, 3 km 41° NE Andranomay, 11.5 km 147° SSE Anjozorobe, 1300 m, 5-13. XII. 2000, 18°28’24”S, 47°57’36”E, Fisher, Griswold et al., montane rainforest, malaise trap, BLF2375 (CAS).

Etymology. The specific epithet is derived from the Latin words *bis* meaning twice and *macula* meaning spot, referring to the two spots on the forewing.

2) *acuta* group

(*U. acuta* sp. nov., and *U. trishula* sp. nov.)

Diagnosis. Ocelli located anterad of line between anterior eye angles, crown with anterior margin narrowly pointed, interocular width less than half of the crown median length. Male (Figs. 6.4A-L) pygofer in lateral view with slightly truncate or round posterior margin and with dorsally directed elongate ventral process, in dorsal view with sclerotization confined to its anterior margin; subgenital plate without two apical setae in addition to series of preapical macrosetae on ventral surface; style without preapical tooth; male abdominal 3S apodeme reduced. Female genitalia with first valvula in lateral aspect (Figs. 6.5G, H) broad at base and gradually tapered to acute apex, with spiral screw-like sculpturing confined to apical third. Second valvula in lateral aspect (Figs. 6.6G, H) expanded near base, then abruptly narrowed beyond middle and tapered to acute apex. Ducts sparse, inconspicuous, dorsal angulate teeth originating near middle and extending to apex. Basal teeth big and widely spaced, distal ones small and closely spaced. Seventh sternum with caudal margin produced medially.

***Ujna trishula* sp. nov.**

Figs. 6.2M, N; Figs. 6.4A–F

Diagnosis. This species may be separated by the following: forewing with Ra located distad of base of third apical cell; male pygofer with elongate process extending beyond its posterior margin, style apophysis with abruptly curved ventral margin beyond middle, aedeagus without processes.

Length of male, 3.8 mm.

Coloration. Crown, pronotum, mesonotum, and scutellum black. Crown with inverted anchor-shaped white marking and forewing with white marking near apical margin and apex of cubital vein. Face, legs creamy white. Forewing with hyaline spot on costal margin near apex.

Structure. As in *U. flavidipes*, except anterior margin of crown narrowly produced, forewing with appendix uniformly wide throughout its length, base of Ra located distad of base of third apical cell, length of second apical cell more than two thirds of that of first apical cell.

Male Genitalia. Pygofer without macrosetae, posterior margin truncate, with elongate ventral process extending beyond posterior margin. Subgenital plate gradually tapered near apex, with series of macrosetae on ventral surface from middle to apex, without pair of long apical setae. Style elongate, without preapical tooth, in lateral view ventral margin of apophysis abruptly curved beyond middle, apex acute. Aedeagus elongate, without processes.

Female. Unknown.

Materials Examined. *Holotype* male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 19 – 26. II. 2002, 21°15’3”S, 47°24’25”E, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-17 (CAS).

Etymology. The specific epithet is derived from the Sanskrit word *Trishul* meaning three spear, refering to the inverted marking on the crown.

***Ujna acuta* sp. nov.**

Figs.6.2O, P; Figs. 6.4G–L; Figs. 6.5G, H; Figs. 6.6G, H

Diagnosis. This species is similar to *U. trishula* but differs in having the male pygofer with a short slightly sinuate process not extending beyond the posterior margin, a uniformly wide style apophysis and the aedeagus with a pair of apical processes.

Length of male, 3.6 mm; female, 3.8 mm.

Coloration. As in *U. trishula*. Crown with marking as in *U. trishula* except pair of lateral white linear markings not joining median line.

Structure. As in *U. flavidipes* except anterior margin of crown narrowly produced, forewing with appendix uniformly wide throughout length, base of Ra located in line or basad of third apical cell, length of second apical cell more than two thirds that of first apical cell.

Male Genitalia. Pygofer with or without macrosetae, posterior margin rounded, with elongate acute slightly sinuate ventral process not extending beyond posterior margin. Subgenital plate gradually tapered near apex, with series of macrosetae on ventral surface from base to apex, without pair of long apical setae. Style elongate, without preapical tooth, in lateral view

apophysis uniformly wide throughout its length, apex acute. Aedeagus elongate, with pair of dorsal apical processes.

Female Genitalia. As described for *acuta* group**.**

Materials Examined. *Holotype* male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower at forest edge, 1130 m, 17 – 27. VII. 2003, 21°15’3” S, 47°24’25”E, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-67 (CAS). *Paratypes*: 1 male, same data as holotype except Talatakely, 900 m, 9-19. I. 2001, 21°15’S, 47°25’12”E, D. H. & K. M. Kavanaugh, R. L. Brett, E. Elsom, F. Vangas, malaise trap (CAS); 1 male, MADAGASCAR: Toamasina Province, botanic garden near entrance to Andasibe National Park, 1025 m, 8 – 16. X. 2001, 18°55’34”S, 48°24’28”E, M. Irwin, R. Harin'Hala, malaise trap - tropical forest, MA-01-08B-15 (INHS); 2 males, 1 female, MADAGASCAR: Toamasina Montagne d'Anjanaharibe, 19.5 km 27° NNE Ambinanitelo, 1100m, 12-16. III. 2003, 15°10’42”S, 49°38’06” E, Fisher, Griswold et al. malaise trap, in montane rainforest, BLF8149 (CAS, INHS).

Etymology. The specific epithet is derived from the Latin word *acuo* meaning pointed, referring to the pointed pygofer process.

3) *rostrata* group

(*U. rostrata* sp. nov., and *U. alba* sp. nov.)

Diagnosis. Ocelli located slightly anterad of line between anterior eye angles, crown with anterior margin broadly rounded, interocular width more than half of the crown median length. Male (Figs. 4M-X) pygofer in lateral view with pointed or round posterior margin and with mesally directed short apical process, in dorsal view with broad sclerotization extending to anterior margin of membranously attached anal segment; subgenital plate without two apical setae in addition to series of macrosetae on ventral surface, style without preapical tooth, male abdominal 2S apodeme well developed, cylindrical, extending beyond fourth segment. Female (Figs. 5I-L) first valvulae dorsal sculpturing finely to coarsely dentate; ventral area with strigate sculpturing. Second valvulae (Figs. 6I-L) in lateral aspect elongate, blade shaped, dorsal margin without notch, ducts inconspicuous, dorsal teeth crenulate or angulate. Seventh sternum with caudal margin produced or truncate.

***Ujna rostrata* sp. nov.**

Figs. 6.2Q, R; Figs. 6.4M–R; Figs. 6.5I, J; Figs. 6.6I, J

Diagnosis. This species may be separated by the following combination of characters: body light brown, posterior margin of male pygofer pointed, style apophysis abruptly curved beyond middle, aedeagus elongate with apical process arising near ventral margin, first valvula of female genitalia with hexagonal shaped fine sculpturing confined to apex, second valvulae with dorsal teeth crenulate, extending to ventral margin.

Length of male, 4.1 mm; female, 4.7–4.8 mm.

Coloration. Crown light brown with median white spot, in some specimens extending to hind margin, and a pair of white lateral lines extending to ocellus. Pronotum, mesonotum, and scutellum light brown without markings. Face and legs white without markings. Forewing translucent with light colored conspicuous veins.

Structure. Crown broadly rounded in dorsal aspect, ocelli located anterad of the line between anterior eye angles. Laterofrontal suture extending onto crown and attaining ocelli. Face convex, in some specimens with slight depression near transclypeal suture in lateral view, muscle impressions indistinct. Pronotum with indistinct striations on posterior half, median length longer than that of crown. Forewing with appendix uniformly wide throughout length; veins distinct; with four apical cells, base of Ra located proximad of base of third apical cell; length of second apical cell two-thirds that of first apical cell; costal area opaque proximad of apical cells, in some specimens apically bordered by vein.

Male Genitalia. Pygofer without macrosetae, posterior margin slightly pointed, with mesally directed apical process on posterior margin. Subgenital plate in lateral view with dorsal margin near base expanded, then gradually tapered towards rounded apex, with series of macrosetae closer to dorsal margin on ventral surface from base to apex. Style elongate, without preapical tooth, in lateral view apophysis abruptly curved beyond middle, apex acute. Aedeagus elongate, in lateral view with pair of dorsally directed preapical processes arising near ventral margin of shaft.

Female Genitalia. First valvulae in lateral aspect narrow, elongate, with fine dentate, hexagon shaped sculpturing confined to apex. Second valvulae in lateral aspect elongate, blade shaped, dorsal margin without the cleft. Ducts few in number, inconspicuous. Teeth as in *U. flavidipes*.

Materials Examined. *Holotype* male, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Belle Vue at Talatakely, 1020 m, 14 – 23. IV. 2002, 21°15’59”S, 47°25’12”E, M. Irwin, R. Harin'Hala, malaise, secondary tropical forest MA-02-09C-25 (CAS). *Paratypes*: 2 females same data as holotype (CAS, INHS); 1 female, same data as holotype except 15 – 22. XI. 2001, MA-02-09C-03 (CAS); 2 females, same data as holotype except 22 – 28. XI. 2001, MA-02-09C-04; 2 females, same data as holotype except 21 – 28. I. 2002, MA-02-09C-13 (CAS, INHS); 1 female, same data as holotype except 19 – 26. II. 2002, MA-02-09C-17 (CAS, INHS); 2 females, same data as holotype except 31. III – 7. IV. 2002, MA-02-09C-23 (CAS, INHS); 1 female, same data as holotype except 28. IV. – 5. V. 2002, MA-02-09C-27 (CAS); 2 females, same data as holotype except 24. VII– 4. VIII. 2002, MA-02-09C-36 (CAS, INHS); 2 males, same data as holotype except 23 – 28. IV. 2002, MA-02-09C-26 (CAS, INHS); 1 male, 2 females, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, radio tower, at forest edge, 1130 m, 15 – 21. XII. 2001, 21°15’3” S, 47°24’25”E, M. Irwin, R. Harin'Hala, malaise, mixed tropical forest, MA-02-09B-07 (CAS, INHS); 1 female, same as previous except16. X. – 8 XI. 2001, MA-02-09B-01 (INHS); 1 male, 1 female, same as previous except 14 – 21. I. 2002, MA-02-09B-12 (CAS, INHS); 1 female, same as previous except 24. XII. 2001 – 2. I. 2002, MA-02-09B-09 (CAS); 1 male, 2 females, MADAGASCAR: Province Fianarantsoa, Parc National Ranomafana, Vohiparara, at broken bridge, 1110 m, 28 XI. – 6 XII. 2001, 21°13’34”S, 47°22’11”E, M. Irwin, R. Harin'Hala, malaise trap in high altitude rainforest, MA-02-09A-05 (CAS, INHS); 2 females, same as previous except 4 – 12. II. 2002, 21°13’34”S, 47°22’11”E, M. Irwin, R. Harin'Hala, malaise trap in high altitude rainforest, MA-02-09A-15 (CAS, INHS); 1 male, 2 females, Madagascar: Province Fianarantsoa, Parc National Ranomafana, Talatakely, 900 m, 21°15’S, 47°25’12”E, 9-19. I. 2001, Malaise trap, D. H. & K. M. Kavanaugh, R. L. Brett, E. Elson and F. Vargas collector, CASENT 3003185, CASENT 3003186, CASENT 3003171 (CAS, INHS); 1 female, MADAGASCAR: Province Fianarantsoa, Ranomafana, JIRAMA water works, 690 m, 21 – 28. I. 2002, 21°14’54”S, 47°27’7”E, M. Irwin, R. Harin'Hala, malaise trap near river, MA-02-09D-13 (CAS).

Etymology. The specific epithet is derived from the Latin word *rostratus* meaning having a beak, referring to the beak shaped male style.

Ujna alba **sp. nov.**

Figs. 6.2S, T; Figs. 6.4S–X; Figs. 6.5K, L; Figs. 6.6K, L

Diagnosis. This species may be separated by the following combination of characters: body creamy white, posterior margin of male pygofer rounded, style apophysis almost uniformly wide, aedeagus short and stout with apical process arising from dorsal margin, first valvula of female genitalia with coarse dentate sculpturing in distal two thirds, second valvulae with dorsal dentate teeth not extending to ventral margin.

Length of male, ~3.5 mm; female, ~3.75 mm.

Coloration. Body creamy white except for the black markings at apex of clavus and apical border of the forewing.

Structure. As in *U. rostrata.*

Male Genitalia. Pygofer without macrosetae, posterior margin rounded, with short mesally directed apical process on posterior margin. Subgenital plate as in *U. rostrata*, but with macrosetae widely scattered on ventral surface. Style elongate with apophysis uniformly wide throughout length. Aedeagus short and stout, with pair of apical processes.

Female Genitalia. First valvulae in lateral aspect narrow, elongate, with coarse dentate sculpturing in distal two thirds. Second valvulae in lateral aspect elongate, blade shaped, dorsal margin without notch. Ducts fewer in number, inconspicuous. Dorsal teeth dentate, widely spaced near apex, confined to dorsal margin, not extending to ventral margin.

Materials Examined. *Holotype* male, MADAGASCAR: Province d'Antsiranana, Montaigne Francais, 150 m, 6 – 20. III. 2001, 12°19'30"S, 49°20'E, M. Irwin, R. Harin'Hala, malaise, forested limestone ridge, MA-01-06-07 (CAS). *Paratypes*: 1 female, same data as holotype (INHS).

Etymology. The specific epithet is derived from the Latin word *albus* meaning white, referring to the white body of the species.

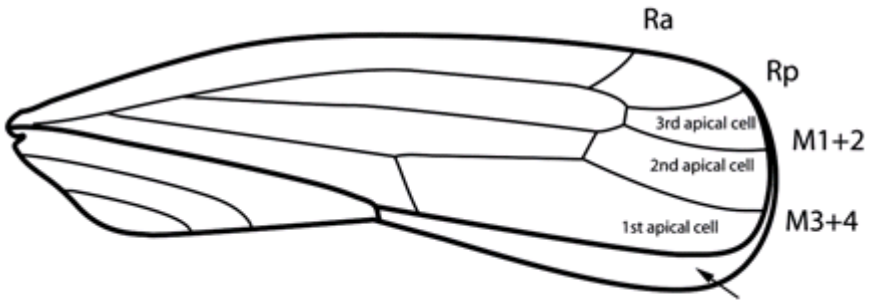

Figure 6.1 Forewing of *Ujna affinis* sp. nov. Arrow indicates appendix.

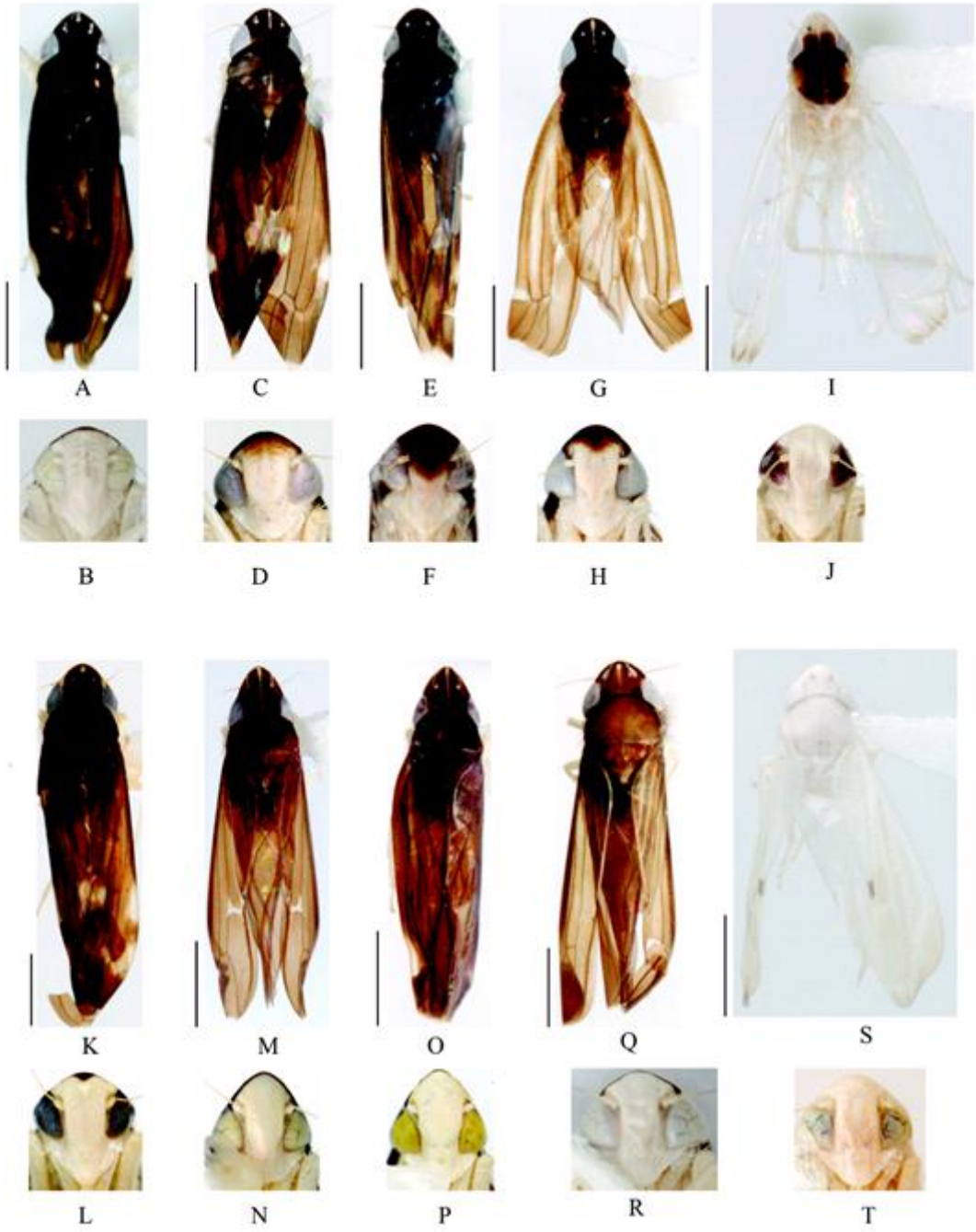

Figure 6.2 *Ujna* spp., dorsal habitus and face, A-T; A, B, *Ujna flavidipes* Distant; C, D, *Ujna affinis* sp. nov.; E, F, *Ujna variabilis* sp. nov. (morph 1); G, H, *Ujna variabilis* sp. nov. (morph 2); I, J, *Ujna variabilis* sp. nov. (morph 3); K, L, *Ujna bimaculata* sp. nov. ; M, N, *Ujna trishula* sp. nov.; O, P, *Ujna acuta* sp. nov.; Q, R, *Ujna rostrata* sp. nov.; S, T, *Ujna alba* sp. nov. Scale bar, 1mm.

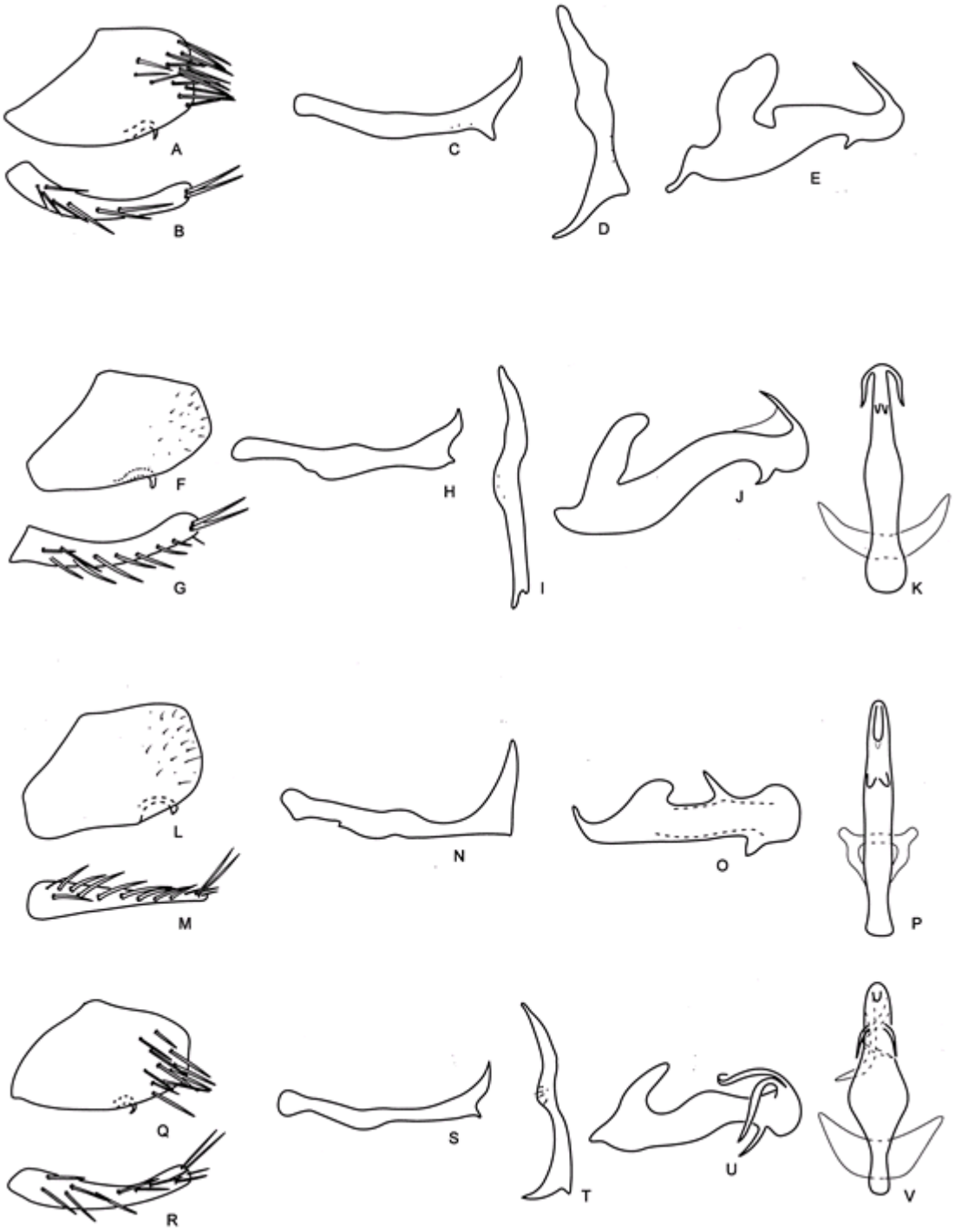

Figure 6.3 Male genitalia of *Ujna* spp. Pygofer in lateral view (A, F, L, Q), subgenital plate in lateral view (B, G, R) and ventral view (M), style in lateral view (C, H, N, S), style in dorsal view (D, I, T), aedeagus in lateral view (E, J, O, U) and ventral (K, P, V) views. A–E, *Ujna flavidipes* Distant; F–K, *Ujna affinis* sp. nov.; L–P, *Ujna variabilis* sp. nov.; Q–V, *Ujna bimaculata* sp. nov. [Subgenital plates of all species included in Figure 3 have similar shape. Fig. 3M is included to show shape of subgenital plate in ventral view.]

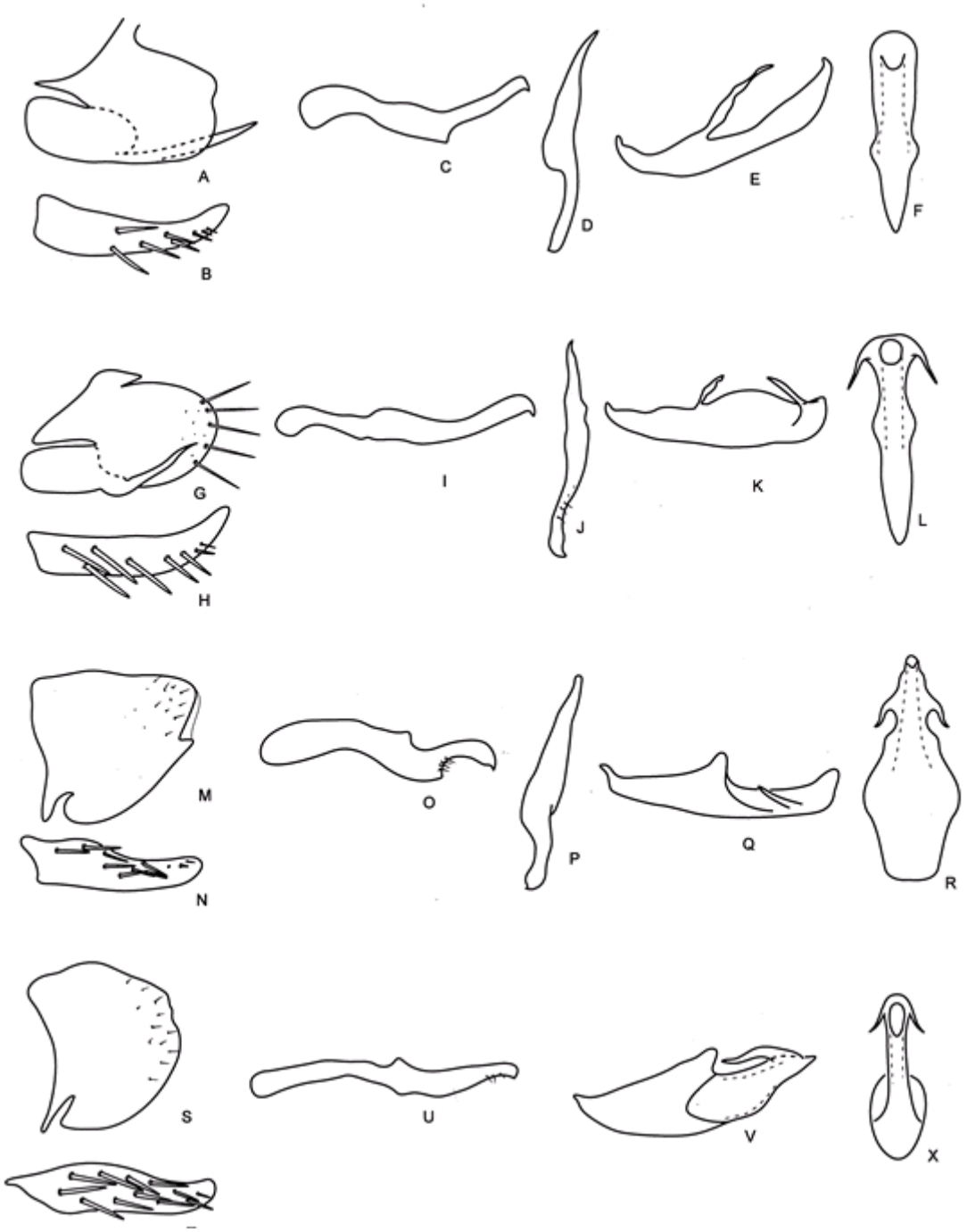

Figure 6.4 Male genitalia of *Ujna* spp. Pygofer in lateral view (A, G, M, S), subgenital plate in lateral view (B, H, N, T), style in lateral view (C, I, O, U), style in dorsal view (D, J, P), aedeagus in lateral view (E, K, Q, V) and ventral (F, L, R, X) views. A–F, *Ujna trishula* sp. nov.; G–L, *Ujna acuta* sp. nov.; M–R, *Ujna rostrata* sp. nov.; S–W, *Ujna alba* sp. nov.

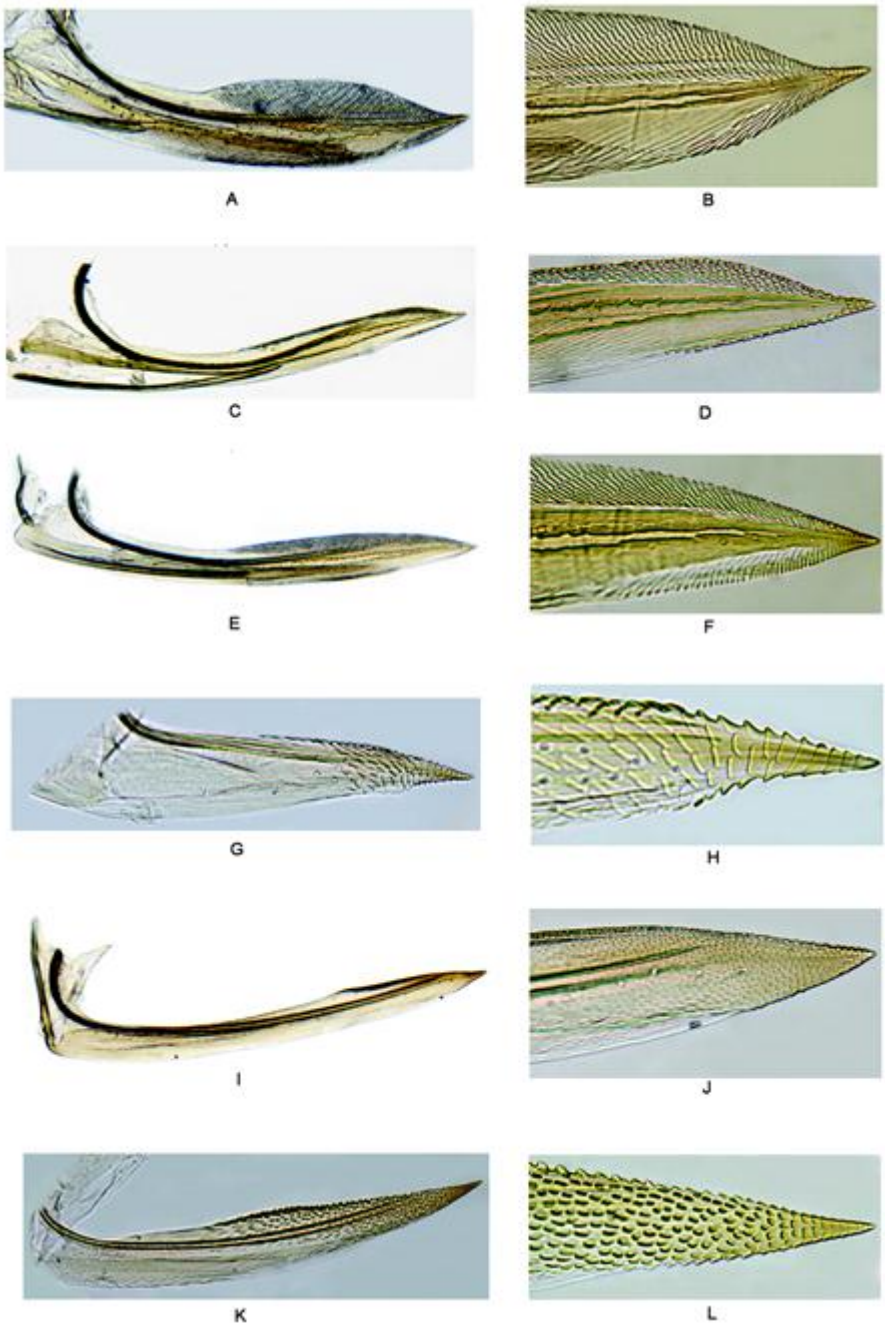

Figure 6.5 First valvulae of female. left- lateral view, right- detail of sculpturing. A–B, *Ujna flavidipes* Distant; C–D, *Ujna variabilis* sp. nov.; E–F, *Ujna bimaculata* sp. nov.; G–H, *Ujna acuta* sp. nov.; I–J, *Ujna rostrata* sp. nov.; K–L, *Ujna alba* sp. nov.

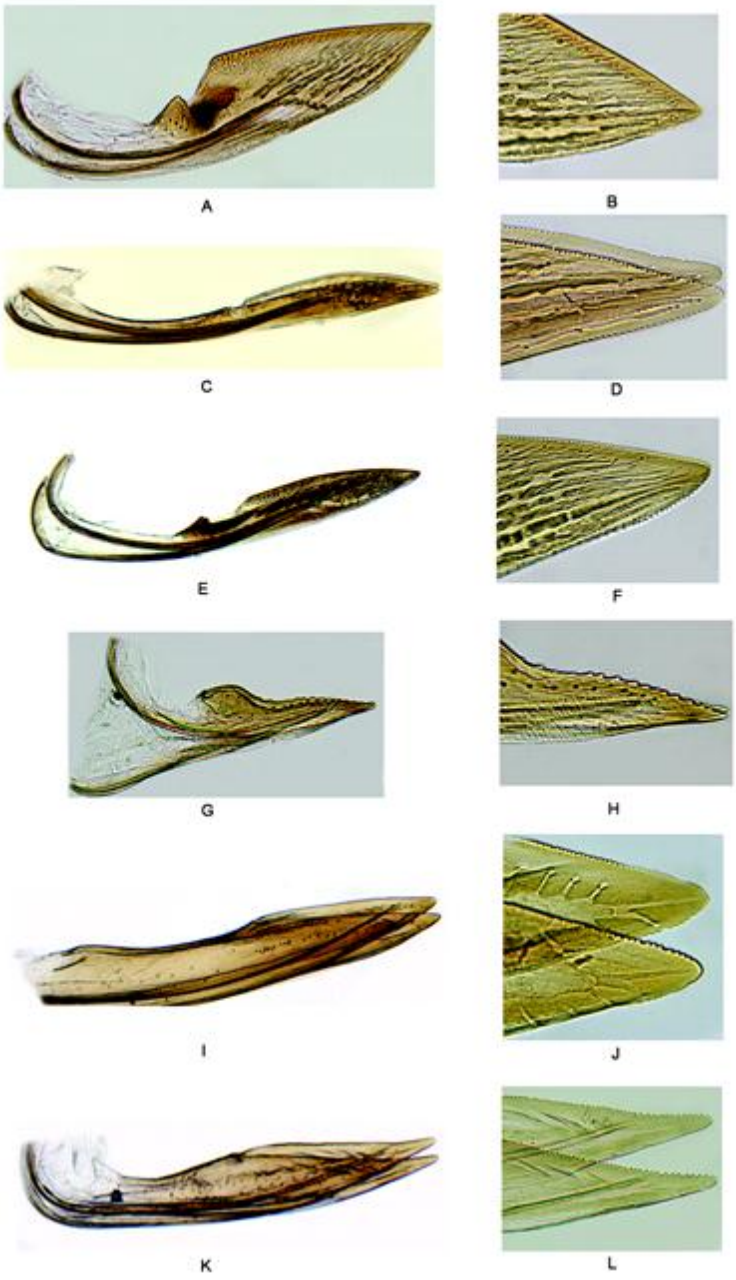

Figure 6.6 Second valvulae of female. left- lateral view, right- detail of sculpturing. A–B, *Ujna flavidipes* Distant; C–D, *Ujna variabilis* sp. nov.; E–F, *Ujna bimaculata* sp. nov.; G–H, *Ujna acuta* sp. nov.; I–J, *Ujna rostrata* sp. nov.; K–L, *Ujna alba* sp. nov.

Chapter 7

Summary and Conclusions

The main objective of my dissertation was to elucidate the historical biogeography of leafhoppers in Madagascar. Apart from this main objective, I also completed taxonomic revisions of two widely distributed leafhopper groups in Madagascar; the endemic idiocerine genus, *Nesocerus* and the subfamily Mileewinae. Thus, my dissertation is divided into two main categories: 1) Biogeographical history of Malagasy leafhoppers and 2) Taxonomic revisions. These topics are discussed separately below.

1) *Biogeographical history of leafhoppers in Madagascar*.

Cicadellidae is one of the most diverse insect families in Madagascar. Considering its diversity, three independent lineages were selected so that their biogeographical histories can be compared to draw general conclusions about the timing of diversification of this family. Phylogenetic analysis based on molecular data was used to examine relationships of the Malagasy leafhopper fauna with those of other continents. The resulting phylogenies and Bayesian molecular dating methods were used to estimate the time of divergence of each lineage in order to test biogeographical hypotheses relating to geological events.

The subfamilies, Iassinae, Idiocerinae and Cicadellinae were chosen as focal taxa. DNA sequences from the following gene regions were used for phylogenetic analyses based on their phylogenetic informativeness within each group: D2, D8, D9-10 regions of 28S rRNA (28S), Histone (H3) and 12S rRNA (12S) for Iassinae; D2, D6, and D8 regions of 28S, Histone H3 for Idiocerinae and D8 of 28S, H3 and Cytochrome Oxidase II for Cicadellinae. Phylogenetic analyses were performed based on Parsimony, Maximum Likelihood and Bayesian approaches. These analyses recovered congruent topologies for each dataset. Bayesian divergence time analysis using soft-bound fossil calibrations were estimated. Results suggest that the biogeographical history of Malagasy leafhoppers can be explained by a combination of Cretaceous vicariance and Cenozoic dispersals. All three lineages have endemic Malagasy groups with origins that are consistent with a scenario of Cretaceous vicariance, followed by endemic species radiation within Madagascar coinciding with the putative spread of eastern rainforests. An ‘Out of Madagascar’ dispersal to the New World was clearly evident in one of

the subfamilies, Cicadellinae, while in the other two subfamilies the closest non-Malagasy relatives of the endemic group remained unclear. Given our current knowledge of biogeographic history of Malagasy insects, this is the first study that revealed this pattern based on three different lineages using both phylogenetic inference and divergence time estimates. Two prior studies revealed similar patterns in butterflies (Torres et al., 2001; Zakarov et al., 2004), but neither of these studies used phylogenetic dating to assess the geologic timing of divergence events. Also, the present study revealed a combination of South American, Asian and African affinities of the Malagasy leafhopper fauna rather than just African relatedness, which is the most predominant pattern in previous insect studies. With regard to Cenozoic dispersal, both Asian and African affinities were found in this study and can be best explained by transoceanic dispersal.

2) Taxonomic revisions

Twenty-nine new species in the endemic idiocerine genus, *Nesocerus* and seven new species in subfamily Mileewinae were discovered and described in this study. Identification keys to all known species of these two groups were also provided. In *Nesocerus*, morphological phylogenetic analysis supported the monophyly of this genus. Two sister clades within this genus, one restricted to the dry western region and the other widely distributed in eastern humid forests were recovered in this analysis. In Mileewinae, three species groups were recognized for the Madagascar fauna. An identification key to the known genera of the subfamily was also given. Extensive variation of female genitalia within this subfamily and their taxonomic utility were reported for the first time.

Future work

Sister group relationships of Malagasy endemic clades of subfamilies, Iassinae and Idiocerinae should be further explored by additional taxon sampling. In Iassinae, more resolution at the base of the resulting tree may be achieved by addition of sequence data for more nuclear genes. Although several attempts to obtain sequences of additional nuclear genes during my dissertation were unsuccessful, this may be due to poor preservation of available leafhopper samples from Madagascar. Relatedness of Malagasy endemic fauna with those of other continents may be better resolved with addition of materials from less sampled regions,

especially southern India and Sri Lanka as the present study suggests divergence of early lineages during the existence of the Indo-Madagascar block.

The oldest fossils of iassines and cicadellines used in this study are from Dominican amber and idiocerines are completely unknown from fossils. Thus, discovery of additional fossils of these groups from earlier geologic time periods remain integral in improving precision and accuracy of divergence time estimates, thereby improving stability of underlying assumptions made during this study.

The present study revealed endemic leafhoppers diversified extensively at the genus/species level within Madagascar. A similar pattern is evident in the leafhopper faunas of other tropical forests, too, where diversification is more profound at lower taxonomic levels. Thus, large-scale biogeographic patterns in leafhoppers can be result of isolation of relict lineages representing higher taxonomic levels in different continents prior to Gondwanan separation, followed by subsequent diversification at lower taxonomic levels. The other processes that might have contributed to the biogeographical patterns of leafhoppers worldwide are extinction that may explain absence of many taxonomic groups in different continents and dispersal at different spatial and temporal scales. An integrative approach of phylogeny-based biogeography and ecology (Weins and Donoghue, 2004) can help improve our understanding large-scale biogeographical patterns.

Broader context

Reliable taxonomy and accurate species distribution data constitutes key tool of conservation planning. Taxonomic study can provide information required to identify areas based on local endemics and taxa of high conservation importance (eg: genera or families). Madagascar is a top global conservation priority due to its high rate of deforestation. It has been estimated that Madagascar lost 40% of its forest cover between 1950 and 2000 (Harper et al 2007). This is alarming because 90% of the Madagascar fauna depends on forest habitat. Different ecosystems within Madagascar have been identified as highly fragmented and degraded (Ganzhorn et al 2001). During recent decades several conservation efforts have been made leading to establishment of protected areas. However, these protected areas encompass only a portion of Madagascar biodiversity leaving countless species outside these areas unprotected (Schatz et al 2000). Identification of these priority areas has been mainly based on a

few threatened species of vertebrates and plants. Kremen et al (2008) emphasized the importance of using a multi-taxon approach in prioritization of conservation areas to increase resolution within hotspots.

The present study reveals many new species, species distribution data and endemic clades, both at species and generic levels in phylogenetic analyses. Many of the new species are recorded from the three unique ecosystems, eastern littoral forest, western dry deciduous forest and evergreen forests of the high plateau that are considered critically vulnerable (Ganzhorn et al 2001). The new data provided by this study will hopefully contribute to the development of more objective means of identifying and protecting priority areas based on multiple taxa, including lesser-known but ecologically important insect groups like leafhoppers.

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